IMMUNOHISTOCHEMICAL EXPRESSION OF MATRIX METALLOPROTEINASE-2 (MMP-2) IN INGUINAL HERNIA

DISSERTATION

SUBMITTED FOR

M.D PATHOLOGY (Branch III)

THE TAMILNADU DR M.G.R MEDICAL UNIVERSITY

DEPARTMENT OF PATHOLOGY

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

PEELAMEDU, COIMBATORE-641004

TAMILNADU, INDIA

MAY 2018

DECLARATION

I solemnly declare that the dissertation titled "IMMUNOHISTOCHEMICAL EXPRESSION OF MATRIX METALLO-PROTEINASE-2 (MMP-2) was done by me from Nov. 2015 to June 2017 under the guidance and supervision of Professor Dr.S.Shanthakumari M.D., Pathology.

This dissertation is submitted to the Tamilnadu Dr.MGR Medical University towards the partial fulfilment of the requirement for the award of MD Degree of Pathology (Branch III).

Place: Coimbatore

Date : Dr.Sruti Mary Joseph

CERTIFICATE

This is to certify that the dissertation work entitled **"IMMUNOHISTO CHEMICAL EXPRESSION OF MATRIX METALLOPROTEINASE-2 (MMP-2) IN INGUINAL HERNIA"** submitted by Dr. Sruti Mary Joseph is a work done by her during the period of study in the Department of Pathology, PSGIMS&R from June 2015 to May 2018. This work was done under the guidance of Dr. S. Shanthakumari, Department of Pathology.

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To
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Ref: Project No.15/395

Date: December 29, 2015

Dear Dr Shruti Mary Joseph,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 18.12.2015 to conduct the research study entitled *"Immunohistochemical expression of matrix metalloprotein-2 (MMP-2) in inguinal hernia"* during the IHEC meeting held on 24.12.2015.

The following documents were reviewed and approved:

1. Project Submission form
2. Study protocol (Version 1 dated 18.12.2015)
3. Confidentiality statement
4. Application for waiver of consent
5. Data collection tool (Version 1 dated 18.12.2015)
6. Current CVs of Principal investigator, Co-investigators
7. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 24.12.2015 at IHEC Secretariat, PSG IMS & R between 10.00 am and 11.00 am:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mr. R. Nandakumar	BA., BL	Legal Expert, Chairperson	Male	No	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Dr. S. Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
4	Dr D Vijaya	M Sc., Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

PSG Institute of Medical Sciences & Research
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November 8, 2016

To
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The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore - 4, has reviewed your proposal on 4th November 2016 in its expedited review meeting held at IHEC Secretariat, PSG IMS&R, between 10.00 am and 11.00 am, and discussed your request to renew the approval and to include two co-investigators for the study entitled:

"Immunohistochemical expression of matrix metalloprotein-2 (MMP-2) in inguinal hernia"

The following documents were received for review:

1. Request for renewal dated 03.11.2016
2. Status report

After due consideration, the Committee has decided to renew the approval for the above study.

The members who attended the meeting held on at which your proposal was discussed, are listed below:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mr R Nandakumar (Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Dr S Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
4	Dr Sudha Ramalingam	MD	Epidemiologist, Ethicist Alt. member-Secretary	Female	Yes	Yes
5	Dr D Vijaya	M Sc., Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The approval is valid for one year (29.12.2016 to 28.12.2017).

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Yours truly,

SECRETARY
PSG IMS&R
COIMBATORE-641004.

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

Proposal No. 15/395

URKUND

Urkund Analysis Result

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Submitted By: srutij3@gmail.com
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Thesis_Pirjo_stm_24.9.2014_Urkundiin.docx (D11584179)
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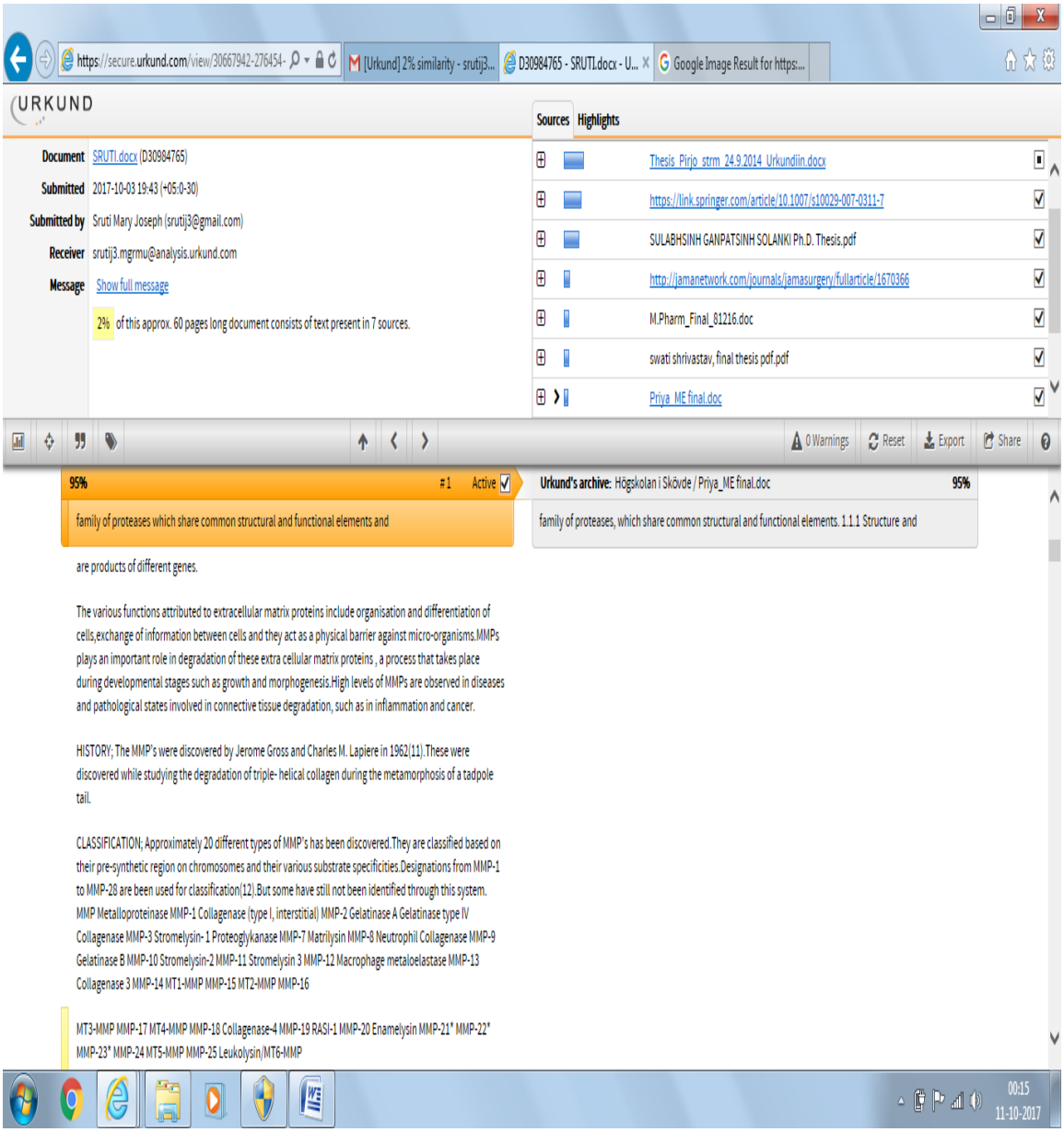
26



are products of different genes.

The various functions attributed to extracellular matrix proteins include organisation and differentiation of cells,exchange of information between cells and they act as a physical barrier against micro-organisms.MMPs plays an important role in degradation of these extra cellular matrix proteins , a process that takes place during developmental stages such as growth and morphogenesis.High levels of MMPs are observed in diseases and pathological states involved in connective tissue degradation, such as in inflammation and cancer.

HISTORY; The MMP's were discovered by Jerome Gross and Charles M. Lapiere in 1962(11).These were discovered while studying the degradation of triple- helical collagen during the metamorphosis of a tadpole tail.

CLASSIFICATION; Approximately 20 different types of MMP's has been discovered.They are classified based on their pre-synthetic region on chromosomes and their various substrate specificities.Designations from MMP-1 to MMP-28 are been used for classification(12).But some have still not been identified through this system. MMP Metalloproteinase MMP-1 Collagenase (type I, interstitial) MMP-2 Gelatinase A Gelatinase type IV Collagenase MMP-3 Stromelysin- 1 Proteoglykanase MMP-7 Matrilysin MMP-8 Neutrophil Collagenase MMP-9 Gelatinase B MMP-10 Stromelysin-2 MMP-11 Stromelysin 3 MMP-12 Macrophage metaloelastase MMP-13 Collagenase 3 MMP-14 MT1-MMP MMP-15 MT2-MMP MMP-16

MT3-MMP MMP-17 MT4-MMP MMP-18 Collagenase-4 MMP-19 RASI-1 MMP-20 Enamelysin MMP-21* MMP-22* MMP-23* MMP-24 MT5-MMP MMP-25 Leukolysin/MT6-MMP

CONTENTS

ACKNOWLEDGEMENT

‘HE is my saviour and my strength.’

Firstly, I would like to thank lord Almighty, for supporting me in all ups and downs and help me complete my dissertation on time.

It gives me immense pleasure to express my sincere gratitude and heartfelt thanks to my guide Dr.S. Shanthakumari, Professor of Pathology, PSGIMS&R for her valuable guidance, immense patience, timely advice and constant support.

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INTRODUCTION

Abdominal wall hernias are regarded as a mechanical problem with a local defect, probably due to weakening of the transversalis fascia. Now a days, hernias are hypothesised as a local manifestation of a systemic disease, which could be proved with an increased expression of MMP-2[(1)].

A close association between inguinal hernia and impaired collagen metabolism are widely studied. Many experiments have proved that a decrease in collagen type I/ collagen type III is found in adult patients with abdominal wall hernias[(2,3)].This finding is further emphasised with the increased risk of hernia in patients with other connective tissue disorders like abdominal aortic aneurysm[(4)], Ehler Danlos syndrome[(5)] and polycystic kidney disease .This decrease in collagen type I /collagen type III is attributed either to an abnormal collagen metabolism or an altered collagen synthesis.

Collagen type I forms a highly stable and extensively cross linked mature form of collagen. On the other hand collagen type III is an unstable and less cross linked collagen synthesised during the early phases of wound healing. As a result of decreased collagen type I /collagen type III ratio, there is weakening of the transversalis fascia and thereby explaining the pathophysiology of inguinal hernia.

In our study, we focus on the imbalanced breakdown of collagen. The cleavage is regulated by the matrix metalloproteinase's (MMPs). The MMPs belong to the family of zinc endopeptidases. More than 20 different MMPs are

known. This study, focuses on increased immunohistochemical expression of MMP-2 in the fibroblasts of skin and transversalis fascia in patients with inguinal hernia and thereby proving that inguinal hernia is a local manifestation of a systemic disease rather than a mere mechanical defect[1].

AIMS & OBJECTIVES

- To establish a causal association between MMP-2 and inguinal hernia
- To test the hypothesis that inguinal hernia is a local manifestation of a systemic disease rather than a mere mechanical defect.

REVIEW OF LITERATURE

EMBRYOLOGY OF INGUINAL CANAL

Inguinal canal forms the pathway of descent of testes through the abdominal wall into the scrotum. They develop in both male as well as female embryos even though the ovaries (except in rare cases) do not pass through the canal.[1]

By the end of second month, the urogenital mesentry attatches testes and mesonephros to the posterior abdominal wall. As the mesonephros degenerates the attatchment serves as mesentry for the gonad. Extending from the caudal pole of testes is a mesenchymal condensation rich in extracellular matrix called the gubernaculum (a ligament). This ligament descends from lower poles of each testes, passes obliquely along the developing abdominal wall and attaches to the labioscrotal swelling which is the future scrotum or labia majora.

Later the processus vaginalis develops on each side, ventral to the gubernaculum, and herniates through the lower abdominal wall along the pouch formed by gubernaculum. The processus vaginalis accompanied by the muscular and fascial layers of the abdominal wall, evaginates into the scrotal swelling, forming the inguinal canal.

The opening produced by processus vaginalis in the transversalis fascia becomes the deep inguinal ring and that in the external oblique aponeurosis forms the superficial inguinal ring. Between these rings is the inguinal canal.

(Ref; 6. T W. Sadler.Urogenial System.In: Langman's Medical Embryology. 10th ed. India:Lipincott Williams & Wilkins; 2006 pg: 253-255)

ANATOMY

INGUINAL CANAL

It is a slit like passage which extends in a downward and medial direction and is situated just above and parallel to the medial half of inguinal ligament. It is approximately 4cm long and extends from the deep inguinal ring to the superficial inguinal ring. It is larger in males than in females.

DEEP (INTERNAL) INGUINAL RING

It is an oval shaped opening in the transversalis fascia and is situated 1.2 cm above the mid inguinal point and immediately lateral to the stem of the inferior epigastric artery. It marks the beginning of the inguinal canal.

SUPERFICIAL (EXTERNAL) INGUINAL RING

It is a triangular shaped opening in the external oblique aponeurosis, with its apex pointing superolaterally and base formed by the pubic crest. The medial and lateral margins reffered to as crura are attached to the pubic symphysis and pubic tubercle respectively. At the apex the two crura are held together by intercrural fibres, which prevent further widening of the superficial ring. It marks the end of inguinal canal and is situated superior to the pubic tubercle.

BOUNDARIES OF INGUINAL CANAL

1.Anterior wall

2. Posterior wall

3. Roof

4. Floor

1. ANTERIOR WALL

In its whole length, its covered by skin, superficial fascia and external oblique aponeurosis. In its lateral one third, its reinforced by the internal oblique muscle.

2.POSTERIOR WALL

The posterior wall is formed along its whole extent by the fascia transversalis, extraperitoneal tissue and parietal peritoneum. It is reinforced by the conjoint tendon along its medial one third.

3.ROOF

Roof is formed by the arched fibres of internal oblique and tranversus abdominis muscles.

4.FLOOR

Its formed by the upper surface of the inguinal ligament and at the medial end by the lacunar ligament.

STRUCTURES PASSING THROUGH THE INGUINAL CANAL

1. Spermatic cord in males and the round ligament of uterus in females. These enter the inguinal canal through the deep inguinal ring and passes out through the superficial inguinal ring.

2. The ilioinguinal nerve enters the canal through the interval between the external and internal oblique muscles and passes out through the superficial inguinal ring.

CONSTITUENTS OF SPERMATIC CORD ARE

1. Vas deferens.

2. Testicular arteries, cremasteric arteries and the artery to the vas deferens.

3. Pampniform plexus of veins.

4. Lymphatics from the testes.

5. Genital branch of genitofemoral nerve, sympathetic nerve plexus around the artery to the ductus deferens and visceral afferent nerve fibres.

6. Remains of processus vaginalis.

COVERINGS OF SPERMATIC CORD INCLUDES

1. INTERNAL SPERMATIC FASCIA : It is derived from the transversalis fascia and covers the spermatic cord, along its whole length.

2. CREMASTERIC FASCIA: It is derived from internal oblique and transverses abdominis muscles and covers the cord below the level of theses muscles.

3. EXTERNAL SPERMATIC FASCIA : It is derived from the external oblique aponeurosis and covers the cord below the superficial inguinal ring.

FASCIA TRANSVERSALIS

The inner surface of abdominal muscles are lined by a fascia, which is separated from peritoneum by extra peritoneal connective tissue.The fascia which lines the inner surface of the transversus abdominis muscle is called fascia transversalis.

EXTENT OF FASCIA TRANSVERSALIS;

Anteriorly, the fascia is adherent to the linea alba above the umbilicus. Posteriorly, it merges with the anterior layer of the thorocolumbar fascia and is continuous with the renal fascia.

Superiorly, the fascia is continuous with the diaphragmatic fascia Inferiorly, the fascia is attached to the inner lip of the iliac crest and to the lateral half of the inguinal ligament. Medially it is attached to the pubic tubercle, pubic crest and the pectineal line.

PROLONGATIONS OF TRANSVERSALIS FASCIA

1. A prolongation of transversalis fascia surrounds the spermatic cord and forms the internal spermatic fascia.

2. A part of the fascia prolongs into the thigh and forms the anterior wall of the femoral sheath.

RELATION TO VESSELS AND NERVES

The major arteries to the abdominal wall and pelvis lie inside the fascia transversalis, while the main nerves are found outside the fascia.

INGUINAL HERNIA

Hernia in Latin means “rupture of a portion of a structure”. Groin region, which lies between the lower abdomen and thigh represents one of the weakest points of the abdominal wall and therefore a common site for abdominal wall hernias.

An inguinal hernia is defined as protrusion of the normal abdominal viscera through a defect or weakness in the fascial or muscular layers, which normally confine them. It occurs because the peritoneal sac enters the inguinal canal either;

- indirectly, through the deep inguinal ring. or
- directly ,through the posterior wall of the inguinal canal.

INCIDENCE

Inguinal hernias are the most common form of abdominal wall hernias, constitutes about 80% of cases, with approximately 800,000 inguinal hernia repairs in the USA in the year.

Males are commonly affected by inguinal hernias, male: female ratio of 7: 1. (Richards et al , 2006).In adult males 65% of inguinal hernias are indirect and 55% are right sided(9).

PARTS OF HERNIA

It consists of

1. sac
2. contents
3. coverings.

SAC: It is the protrusion of peritoneum and comprises of a neck (the narrowed part) and a body (the bigger part)

CONTENTS : Mainly, coils of small intestine or omentum or any other viscera.

COVERINGS : These are the layers of the abdominal wall which are covers the hernia sac.

RISK FACTORS FOR THE DEVELOPMENT OF INGUINAL HERNIA

1. Age > 60yrs
2. Males > Females
3. Respiratory disease
4. Dysuria
5. Obesity
6. Constipation
7. Improper weight lifting
8. Postoperative scar
9. Smoking and alcohol abuse.
10. Raised abdominal pressure as in ascites
11. Infections of the inguinal region.
12. Pregnancy.
13. Congenital connective tissue disorders .
14. Defective collagen synthesis.

ETIOLOGY OF INGUINAL HERNIA

1. CONGENITAL
2. ACQUIRED

1.CONGENITAL

Congenital hernias are the most common cause of inguinal hernia in paediatric age group. Processus vaginalis, which is a thin layer of peritoneum that attaches to the testicles as it descends to the scrotum and forms a sac around it. Normally, the processus vaginalis closes shortly after birth.

If the closure of processus vaginalis is delayed or incomplete, it may stretch and eventually become a hernia. The stretching of processus vaginalis creates an inguinal sac, which allows the organs from abdomen to extend and enter into the sac.

2. ACQUIRED

These include any cause that increases the intra abdominal pressure and thereby weakening the abdominal muscles and the fascia.

TYPES OF INGUINAL HERNIA

1. DIRECT INGUINAL HERNIA

A direct inguinal hernia is a protrusion of abdominal viscera through a weakness in the posterior wall of the inguinal canal, specifically along the Hesselbach's triangle.

The Hesselbach's triangle is bounded by inferior epigastric artery, lateral border of rectus abdominis and inguinal ligament. These type of hernias are called direct as the hernial sac directly protrudes through the inguinal wall,

unlike in indirect hernias where they arise through deep inguinal ring and enters the inguinal canal.

PATHOLOGY

Occurs as a result of weakening of transversalis fascia in the Hesselbach's triangle. These are generally acquired and increases with age.

Mainly seen in elderly with chronic conditions which increases the intra-abdominal pressure. Eg; COPD, bladder outlet obstruction and chronic constipation.

As the raised intra abdominal pressure are transmitted to both sides equally, the direct inguinal hernias are commonly bilateral. These are less commonly associated with obstruction or strangulation as compared to indirect inguinal hernia.

2. INDIRECT INGUINAL HERNIA

Most common form of abdominal wall hernias. It is five times more common than direct inguinal hernias. Mainly occurs as a congenital lesion due to patent processus vaginalis. The hernial sac extends through the deep inguinal ring, anteromedial to the spermatic cord in males (or round ligament in females).

They may also be caused due various acquired risk factors of hernia. These are more prone for obstruction and strangulation compared to that of direct inguinal hernia.

COMPLICATIONS OF INGUINAL HERNIA

1. STRANGULATION

An increased pressure over the hernia contents leads to reduced blood supply to organs or tissues, thereby causing ischemia, cell death or even gangrene. Its a life threatening complication and requires immediate surgery.

2. OBSTRUCTION

The bowel gets obstructed leading to abdominal cramps, absence of defeacation and vomiting.

COMPLICATIONS AFTER SURGERY

These include superficial hematoma, infection, scrotal edema , persistent inguinal neuralgia, local hypoesthesia, ischemic orchitis and recurrences.

(Ref: 7. Krishna Garg, Pragati Sheel Mittal, Mrudula Chandraputta. Abdomen & Pelvis. In: B D Chaurasia's Human Anatomy.Vol 2. 7th ed.India: CBS Publishers; 2016: pg 216-238.

Ref: 8. Richard. L. Drake, A. Wayne Vogl, Adam W.L Mitchell.Anterior Abdominal Wall. In:Gray's Anatomy for Students. 3rd ed. Canada: Elseivier; 2005 pg; 292-302.)

CLASSIFICATIONS OF INGUINAL HERNIA

Several important contributions were made by American, French and German surgeons regarding the classification of inguinal hernias. These

classifications are considered a useful tool for surgeons to decide which type of hernia repair may be the best in individual patients[10].The various classifications include;

- Casten Classification 1967
- Halverson and Mc Vay Classification 1970
- Gilbert Classification 1989
- Nyhus Classification 1991
- Bendavid Classification 1993
- Aachen Classification 1995
- Zollinger Classification 2003

TABLE NO.1 : NYHUS CLASSIFICATION

Type1	Indirect inguinal hernia with a normal ring sac in the canal.
Type 2	Indirect hernia with an enlarged internal ring but the posterior wall is intact; inferior deep epigastric vessels not displaced, sac not in scrotum.
Type 3a	Direct hernia with posterior floor defect only.
Type 3b	Indirect hernia with enlargement of internal ring and posterior floor defect.
Type 3c	Femoral hernia
Type 4	Recurrent hernia

TABLE NO.2 : AACHEN CLASSIFICATION

L	Lateral hernia
M	Medial hernia
Mc	Combined hernia
F	Femoral hernia
I	Hernia orifice < 1.5 cm
II	Hernia orifice 3cm
III	Hernia orifice >3 cm

TABLE NO.3 : MODIFIED TRADITIONAL CLASSIFICATION

(ZOLLINGER 2003)

I	A : Indirect small B : Indirect medium C : Indirect large
II	A : Direct small B : Direct medium C : Direct large
III	Combined
IV	Femoral
O	Other : Any not classified by number above Femoral + indirect / direct Femoral + Indirect + Direct Massive > 8 cm inguinal defect
R	Recurrent

TABLE NO.4 : BENDAVID CLASSIFICATION 1994

Type 1	Anterolateral or indirect
Type 2	Anteromedial or direct
Type 3	Posteromedial or femoral
Type 4	Posterolateral or prevascular
Type 5	Anteroposterior or inguinofemoral

MATRIX METALLOPROTEINASE

INTRODUCTION

Matrix metalloproteinases also known as matrixins are a group of enzymes that regulate cell- matrix composition. These are zinc dependent endopeptidases which are known for their ability to cleave extracellular matrix constituents such as collagen, elastin, casein, as well as non matrix proteins[(11)]. They are a large family of proteases which share common structural and functional elements and are products of different genes.

The various functions attributed to extracellular matrix proteins include organisation and differentiation of cells, exchange of information between cells and they also act as a physical barrier against micro-organisms. MMPs plays an important role in degradation of these extra cellular matrix proteins , a process that takes place during developmental stages such as growth and morphogenesis. High levels of MMPs are observed in diseases and

pathological states involved in connective tissue degradation, such as in inflammation and cancer

HISTORY

The MMP's were discovered by Jerome Gross and Charles M. Lapiere in 1962[11]. These were discovered while studying the degradation of triple-helical collagen during the metamorphosis of a tadpole tail.

CLASSIFICATION

Approximately 20 different types of MMP's has been discovered. They are classified based on their pre-synthetic region on chromosomes and their various substrate specificities. Designations from MMP-1 to MMP-28 are been used for classification[12]. But some have still not been identified through this system.

TABLE NO.5 : GENERAL CLASSIFICATION OF MMP's

MMP	Metalloproteinase
MMP-1	Collagenase (type I, interstitial)
MMP-2	Gelatinase A Gelatinase type IV Collagenase
MMP-3	Stromelysin- 1 Proteoglykanase
MMP-7	Matrilysin
MMP-8	Neutrophil Collagenase

MMP-9	Gelatinase B
MMP-10	Stromelysin-2
MMP-11	Stromelysin 3
MMP-12	Macrophage metaloelastase
MMP-13	Collagenase 3
MMP-14	MT1-MMP
MMP-15	MT2-MMP
MMP-16	MT3-MMP
MMP-17	MT4-MMP
MMP-18	Collagenase-4
MMP-19	RASI-1
MMP-20	Enamelysin
MMP-21*	
MMP-22*	
MMP-23*	
MMP-24	MT5-MMP
MMP-25	Leukolysin/MT6-MMP
MMP-26	Endometase, matrilysin-2
MMP-28	Epilysin

*MMP genes were found on chromosomes, but their function and structure have not been identified yet

TABLE NO.6 : CLASSIFICATION BASED ON PREFERRED SUBSTRATE

MATRIX METALLOPROTEINASE	MMP NUMBER	PREFERRED SUBSTRATE
CLASS I		
1.Interstitial collagenase	1	Fibrillar collagens, type I, II,III
2.Neutrophil (PMN) collagenase	8	Fibrillar collagens, type I,II,III
3.Collagenase 3	13	Fibrillar collagens type I,II,III
CLASS II		
1.Gelatinase A (72kDa)	2	Collagen types IV, V,gelatin
2.Gelatinase B (92kDa)	9	Collagen types IV,V ,gelatin
3.Metalloelastase	12	Elastin
CLASS III		
1.Stromelysin-1	3	Laminin,fibronectin,proteoglycans
2.Stromelysin-2	10	Laminin,fibronectin,proteoglycans
3.Matrilysin (pump)	7	Laminin,fibronectin,proteoglycans
NON –CLASSIFIED		
Stromelysin-3	11	1-antitrypsin
Membrane-type MMP	14	Pro- gelatinase A

STRUCTURE OF MMPs

MMPs are homologous proteins and are classified into six categories based on its substrate recognition and cleavage mechanism. These includes:

1. Collagenases
2. Stromelysins

3. Matrilysins
4. Gelatinases
5. Membrane associated MMPs
6. MMPs with no group designation

MMPs are zinc and calcium dependant endopeptidases, which are synthesised in an inactive form as pro-MMP. These are secreted from cells in its inactive form, so as to prevent MMPs from cleaving essential components in cells. The enzyme is divided into three domains[13]

1. N-terminal propeptide
2. Catalytic domain
3. C-terminal domain

1. N-Terminal Propeptide

N-Terminal propeptide contains approximately 80 amino acids and it ensures enzyme latency. The most important functional amino acid group in N-Terminal propetide is cysteine. This cysteine group interacts with catalytic zinc ions through a thiol group and constitutes cyteine switch. Within the propeptide, there is a highly conserved sequence (Pro-Arg-Gly-Cys-X-Pro-Asp),where the X represents any amino acid. Cleavage of the propeptide triggers proMMP activation[11].

2. C-Terminal domain

C-Terminal domain is also called hemopexin-like domain and is structurally similar to proteins of the hemopexin family[11]. It contains approximately 210 amino acids. This domain has a relatively larger surface area for protein-protein interactions. It is ellipsoid in shape with a propeller-like subdomain, in which each leaf of the propeller is composed of four antiparellel beta sheets and one alpha helix. The first and the fourth leaf are linked by a disulfide bond. The catalytic and C-terminal domain are freely attatched by a flexible proline rich peptide linker (hinge).

The hemopexin-like domain is characteristic for collagenases and is necessary for degradation of specific amino acid sequences in interstitial collagen.

There is a deletion of this domain in MMP-7,MMP-26, MT1,MT2, MT3, MT5 and MT6- MMP. However this doesn't impair their ability to activate proMMP2.

3. CATALYTIC DOMAIN

The catalytic domain consists of 170 amino acids and a zinc binding motif. It consists of five beta sheets, three alpha helices and connecting loops along with two zinc ions and two or three calcium ions. The first zinc ion present in the active site directly participates in the catalytic process.

The catalytic domain of MMPs has a proteolytic activity. MT-MMPs possess this domain, while the matrilysin MMPs do not contain this domain.

OTHER MINOR REGIONS

1. SIGNAL PEPTIDE

It contains about 17-20 amino acids and serves as a signal for secretion into endoplasmic reticulum.

2. FURIN-CLEAVAGE SITE INSERT

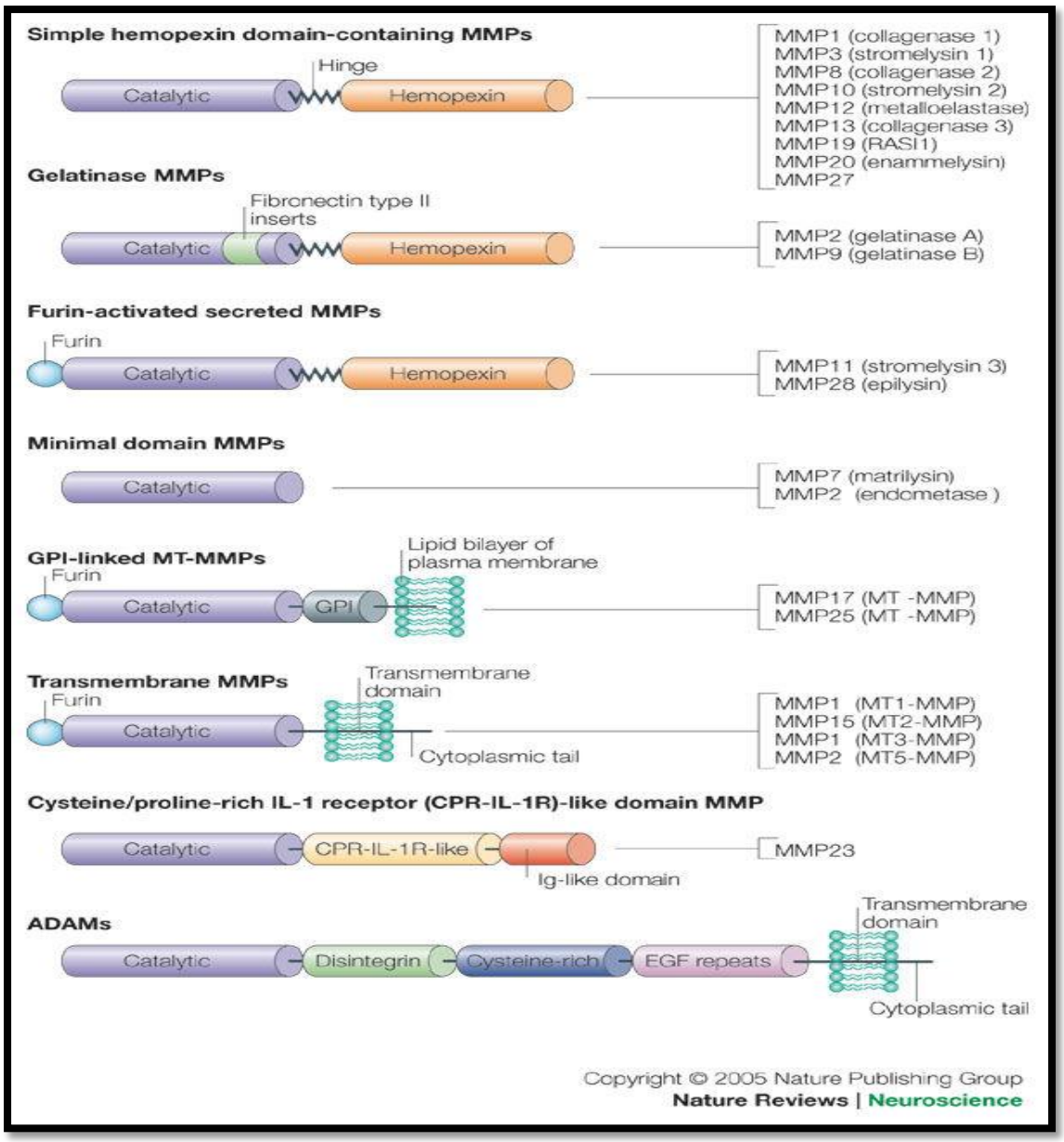
It contains approximately nine amino acids .MMP-11, MMP-14, MMP-15, MMP-16 and MMP-17 possess this sequence.

3. FIBRONECTIN-LIKE REPEATS

These specialised structures helps in binding of enzyme to gelatine and collagen substrates.

4. TRANSMEMBRANE DOMAIN

It contains one hydrophobic chain composed of approximately 25 amino acids. Present in all MMPs except in MT4-MMP and MT6-MMP.



STRUCTURE OF MMP's

COMMON FEATURES OF MMPs

1. These are produced in an inactive state.

2. Contains two zinc atoms,of which oneis in an active site.

3. Contains two calcium ions, which are essential for enzyme stability.

4. The primary structure, typically contains two highly conserved regions. One each in the N-terminal propeptide domain and in the catalytic domain.

5. These are inhibited by specific inhibitors called tissue inhibitors of metalloproteinases.

SUBSTRATE CLEAVING MECHANISM AND SUBSTRATE SPECIFICITY

MMPs can degrade majority of the extracellular matrix components. Other than MMP-11 and MMP-23, most of these enzymes have a broad substrate specificity[11]. These MMPs not only cleave extracellular matrix components, but also act as activators for biologically important molecules.

1. COLLAGENASES

Collagenases includes MMP-1, MMP-8 and MMP-13. These degrade alpha helices of interstitial collagens (type I,II, III). Collagenases differ from other MMPs in that they cleave collagen helices in a native state at neutral PH by a non denaturing mechanism. These significantly differ in their substrate specificities. For eg; Neutrophil collagenases (MMP-8) cleave collagen type I, while interstitial collagenases (MMP-1) cleaves collagen type III .MMP-13 can cleave collagen type I and III, but at a smaller rate than others[11].

2. GELATINASES

These include MMP-2 and MMP-9.They primarily cleave denatured and intact collagen type IV in the basal membranes. They are also capable of cleaving denatured collagen type V,VII, X, XIV, fibronectin, elastin and aggrecan. MMP-2 is also known to cleave native collagen type I, while MMP-9 cleaves a number of other physiological substrates. Substrates containing arginine are more preferred by MMP-9[(14)].

3. STROMELYSINS

These have a relatively broad substrate specificity. Most of these stromelysins, cleave non collagenous extracellular matrix proteins like proteoglycans, glycoproteins, fibronectin and laminin[(11)].Collagen type IV is cleaved in a globular rather than helical conformation by stromelysins. They are also capable of cleacving other MMPs. For eg; stromelysin 2 (MMP-10) can degrade neutrophils collagenases (MMP-8).

4. MEMBRANE ASSOCIATED MMPs

These are also called membrane- type MMPs/ MT-MMPs. These exhibit similar substrate specificity as that of free MMPs. They mainly degrade collagens. The main difference between other MMPs is their association with cell membrane. Other than their location, MT-MMPs also differs in its activity from other types of MMPs. Many of these MT-MMPs have cytoplasmic domains, which are important for cell signalling[(15)].

MMP-14 (MT1-MMP) is about five to seven times less effective in cleaving type I collagen, compared to its analogue MMP-1. However, its eight times more effective in cleaving gelatinases compared to MMP-1.

MT4-MMP has the ability to cleave gelatine and synthetic substrates, but cannot cleave collagen type I and IV, fibronectin and laminin.

MT1-MMP, MT3-MMP and MT4-MMP can cleave inactive forms of MMPs (pro-MMPs), prior to their activation.

5. MACROPHAGE ELASTASE AND OTHER MMPs

Macrophage elatase, also known as MMP-12 shares its ability to cleave elastin along with other MMPs like gelatinases and matrilysins. They also have the ability to degrade other substrates including fibronectin, laminin, collagen, basal membrane, entactin, chondroitin sulphate etc[(10)]. They also enables macrophages to penetrate the basal membrane and thereby initiating an inflammatory reaction. In addition MMP-20 can also degrade tooth enamel, specifically emalogenin.

ACTIVATION AND INHIBITION OF MMPs

Activation of MMPs is mainly through cysteine switch mechanism. This can be achieved by several ways. These include:

1. Treatment with oxidants

2. Treatment with disulfides

3. Treatment with alkylating agents

4. Proteolytic cleavage

5. Usage of agents changing conformation

6. Treatment with silver (I) and mercury(II) ions.

REGULATION OF ACTIVITY OF MMP

The activity of MMPs can be regulated at several different stages, which includes:

1. Transcriptional

2. Post-transcriptional

3. Control at the protein level via their activators or inhibitors.

A normal adult usually harbours very low levels of MMPs, and their production and activity are maintained at a practically undetectable level. The activity of MMPs should be tightly regulated so that they are present within the right cell type and pericellular location at the right time and in the right amount.A loss of their activity control may lead to various diseases[(11)].

The MMPs are mainly regulated at their transcriptional and post transcriptional stages. Their expression becomes elevated, whenever there is a challenge to this system.

REGULATION OF MMP GENE EXPPRESSION

The common genes which are responsible for MMP transcription are inducible and can also be activated by various chemicals like phorbol esters. The factors that may suppress the expression of MMP genes are transforming growth factor beta, glucocorticoids and retinoic acid.

The various transcription binding sites, that are involved in the regulation of MMP genes are;

1. Activator proteins (AP-1 and 2 sites)(15)
2. The polyomavirus enhancer –A binding protein -3(PEA3)
3. NF-KB site
4. STAT site

1. AP- 1 &2 SITES

The AP-1 site is considered as the major mediator in the regulation of MMP genes. As a result, most of the MMP promoters harbour an AP-1 site in their proximal promoter site.

2. PEA3 SITE

The PEA3 site is located adjacent to the AP-1 site. Both AP-1 and PEA3 sites may act co-operatively to promote MMP production in many cancer cells[11]. This results in cancer cell migration and invasiveness.

3.NF-KB BINDING SITE

This pathway is involved in the regulation of a wide range of MMPs. The NF-KB is activated by a number of growth factors and cytokines. These NF-KB mediated MMP activation is involved in many pathological conditions like arthritis, muscular diseases and cancer.

Various inflammatory cytokines like tumor necrosis factor and interleukin -1, indirectly influence the expression of genes for MMP and trigger the ceramide signalling pathway.

This ceramide dependant expression of MMP-1 in human skin fibroblasts is influenced by three distinct MAP kinase pathways. These include;

- ERKI/2
- Stress activated protein kinase
- Protein p38

An important example of an inducible factor, which can increase the expression of MMP-1, MMP-3 and MMP-9 in human dermal fibroblasts is ultraviolet B radiation[(11)].

REGULATION OF MMP ACTIVITY BY CYSTEINE SWITCH

The matrix metalloproteinases are secreted in an inactive form, known as proMMPs. Many of these MMPs are found attached to various extracellular matrix components, which aids in their quick activation.

Different MMPs have affinity for specific extracellular matrix proteins. For eg; MMP-2 binds to extracellular matrix containing elastin.MMP-3 binds to basal membrane and occasionally to collagen fibrils, while MMP-13 binds to proteoglycans, collagen and elastin[11].

The storage forms are different for various MMPs. MMP-8 is stored in specific cell granules. MMP-1 and MMP-3 are constitutively produced by activities of cytokines and inflammatory mediators.

Extracellular activation of enzymes involves two steps:

- In the first step , MMP propeptide is cleaved releasing one Zn (II) ion from the containing cysteine complex. In normal circumstances, there is an interaction between Zn (II) ion and cysteine within the propeptide, which keeps the proMMPs in an inactive state. A mechanism known as CYSTEINE SWITCH, causes dissociation of this complex and leads to release of proMMPs for activation[17].
- The second step involves the cleaving of released proMMP, thereby leading to its activation. This step is assisted by many other MMPs.

INVITRO MECHANISMS OF MMP ACTIVATION

Sodium dodecyl sulphate is the most important agent used for invitro activation of MMP's[18].Other substances like thiol modifying reagents and oxygen free radicals and metallothionein can also cause activation of MMPs. They mainly activate the MMPs via the cysteine switch pathway.

Physicochemical factors like temperature or a decrease in Ph can also trigger MMP activation[11].

TISSUE INHIBITORS OF MATRIX METALLOPROTEINASES (TIMPs)

Tissue inhibitors of matrix metalloproteinases (TIMPs) are naturally occurring proteins that specifically inhibit matrix metalloproteinses. These are the major endogenous regulators of MMPs in the tissue[19].The molecular masses of these proteins ranges from 21 to 30kDa.

Four homologous proteins named as TIMP-1, TIMP-2, TIMP-3 and TIMP-4 have been identified[19]. MMPs bind to these TIMPs at a ratio of 1:1 by forming binary non covalent complexes.

MECHANISM OF ACTION

The TIMPs regulate extracellular matrix turnover and tissue remodelling by forming tightly bound complexes with MMPs[19]. Thereby they maintain a balance between extracellular matrix destruction and formation.

STRUCTURE OF TIMPs

TIMPs have a wedge shaped appearance[19]. These contain six loops held together by six disulfide bonds and are arranged in three knot-like structures. Each TIMP is translated with a 29 amino acid leader sequence, that is cleaved to produce the mature protein.

As in MMPs, TIMPs also contain an N-terminal region and a C-terminal region[19]. Both of these regions enhances the selectivity of inhibition and the binding affinity.

These TIMP proteins may either be secreted extracellularly in a soluble form like TIMP-1,TIMP-2 and TIMP-4.They may also be seen bound to extracellular matrix (TIMP-3).

TYPES OF TIMPs

These are mainly divided into four groups, mainly TIMP-1, TIMP-2, TIMP-3 and TIMP-4.

Based on gene expression, they are divided mainly into two groups;

1. Those which exhibit an inducible expression. (INDUCIBLE)
2. Those which are not inducible or expressed at very low levels. (CONSTITUTIVE)

TABLE NO.7 : CLASSIFICATION OF TISSUE INHIBITORS OF METALLOPROTEINASES

S.NO	INHIBITED MMP	kDa	EXPRESSION	TISSUES	LOCALISATION
TIMP-1	**All except MMP-14**	**28.5**	**Inducible**	**Bones, Ovary**	**Diffusible**
TIMP-2	**All**	**21**	**Constitutive**	**Placenta**	**Diffusible**
TIMP-3	**MMP-1,-2,-3,-9,-13**	**21**	**Inducible**	**Kidney, Brain**	**ECM associated**
TIMP-4	**MMP-1,-2,-3,-7,-9**	**22**	**?**	**Heart**	**Diffusible**

1. TIMP-1

It inhibits almost all known MMP family members. Associates mainly with proMMP-9. The major functions involve inhibition of angiogenesis and potentiating erythroid activity[20].

2. TIMP-2

It inhibits almost all known MMP family members. A high predilection for MT1-MMP and MMP-2 are noted. It is predominantly involved in regulation of MMP-2 activity. It has a bifunctional effect on MMP-2, since MT-MMP mediated proMMP-2 activation requires a tiny amount of TIMP-2. While a greator majority of TIMP-2 is involved in directly inhibiting MMP-2.

3. TIMP-3

It inhibits almost all known MMP family members. It is extracellular matrix associated. Mutation of TIMP-3 is associated with Sorsby's fundus dystrophy. Its major function is promoting apoptosis in many cells both invivo and in vitro.

4. TIMP-4

It inhibits almost all known MMP family members. Its restricted expression suggests tissue specific TIMP function.

OTHER FUNCTIONS OF TIMPs

These include inhibition of the following[20] :

1. Cell invasion invitro,

2. Tumor genesis,

3. Metastasis in vivo and

4. Angiogenesis

TIMP-1 and TIMP-2 has mitogenic activities on a number of cell types. On the other hand over expression of these inhibitors reduces tumor cell growth. Also inhibits basic fibroblast growth factor induced human endothielial cell growth.

MMPs PARTICIPATION IN PHYSIOLOGICAL PROCESSES AND DISEASES

MMPs are involved in both physiological and pathological processes of the body. The MMPs attribute their role in development, reproduction and maintanence of various organs and organ systems[21].

1. DEVELOPMENTAL FUNCTIONS;

These include,

- Blastocyst implantation
- Embryonic development
- Nerve growth
- Growth plate cartilage removal
- Skeletal,bone growth

- Nerve outgrowth
- Enamel maturation
- Primary tooth resorption

2. REPRODUCTIVE FUNCTIONS

- Endometrial cycling
- Graffian follicle rupture
- Luteolysis
- Cervical dilatation
- Postpartum uterine involution
- Mammary gland morphogenesis
- Mammary gland involution
- Rupture of fetal membranes

3. MAINTANENCE FUNCTIONS

These include:

- Remodelling of bone
- Hair follicle cycle
- Wound healing
- Angiogenesis
- Apoptosis
- Nerve regeneration
- Macrophage function

PATHOLOGICAL FUNCTIONS

Other than physiological functions, MMPs also involved in many pathological processus. These are mainly associated with tissue destruction, fibrotic diseases and weakening of matrix[21].

1. TISSUE DESTRUCTION

This function is mainly associated with diseases like:

- Rheumatoid arthritis
- Osteoarthritis
- Cancer invasion
- Cancer metastasis
- Decubitus ulcer
- Gastric ulcer
- Corneal ulceration
- Periodontal disease

2. FIBROTIC DISEASES

These include:

- Liver cirrhosis
- Fibrotic lung disease
- Otosclerosis
- Atherosclerosis
- Multiple sclerosis

3. WEAKENING OF MATRIX

Weakening of extracellular matrix proteins , leads to many diseases including;

- Dilated Cardiomyopathy
- Epidermolysis bullosa
- Aortic aneurysm
- Abdominal wall hernias

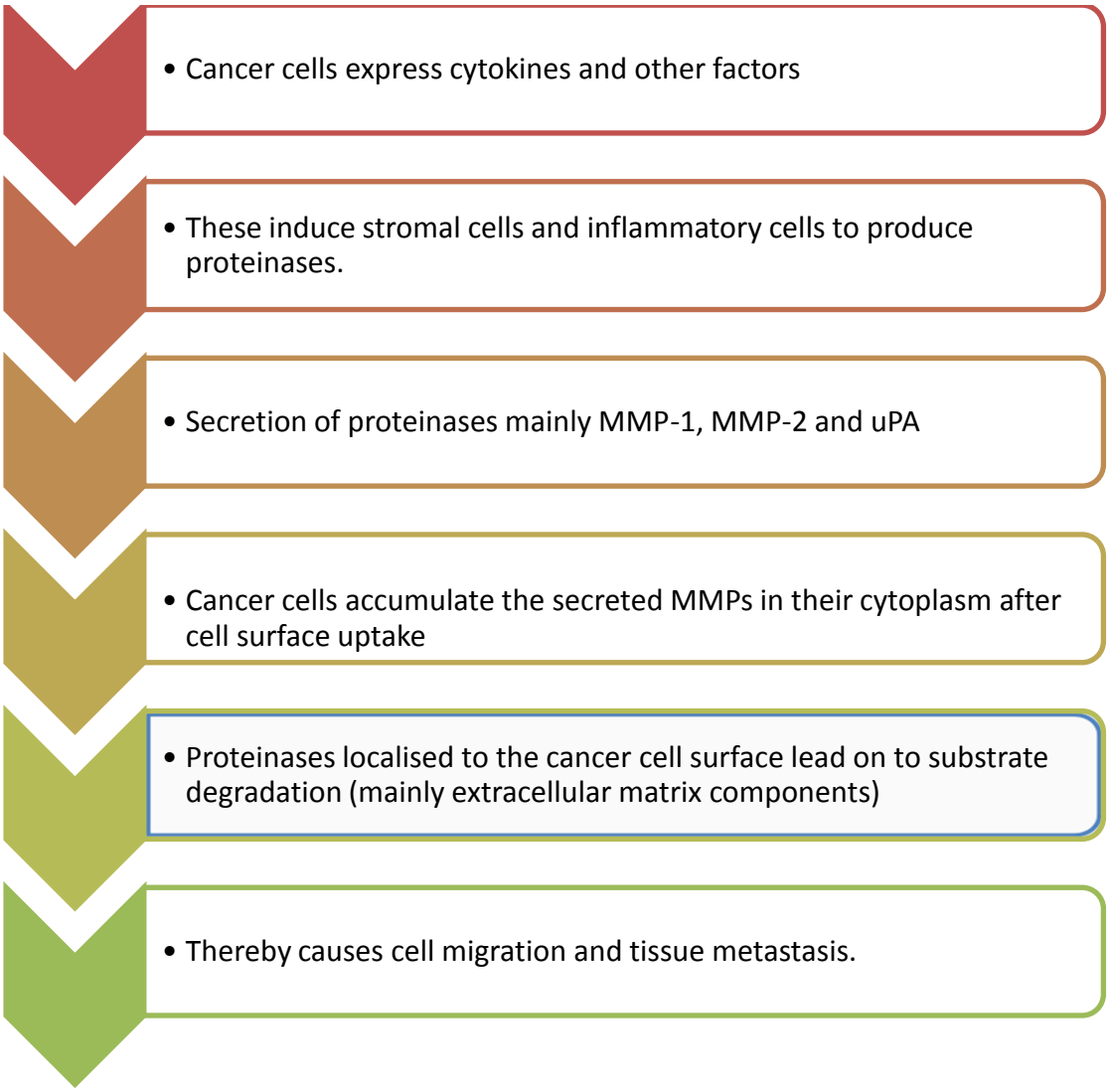
A BRIEF NOTE ON ROLE OF MMPs IN CANCER

MMPs play an important role in carcinogenesis. They predominantly aid in tumor angiogenesis, tissue invasion and metastasis[(22)]. Tissue invasion and metastasis are the two signature features of a malignant neoplasm. Proteolytic degradation of components of basement membrane and extracellular matrix are essential steps involved in tissue invasion. This proteolytic degradation is carried out by proteases and protease-like proteins. Among these proteolytic enzymes, MMPs have been a subject of extensive study.

Association of MMPs are mainly studied in stomach, colorectal, prostate, and head and neck cancers[(22)]. MMPs are involved in degradation of extracellular matrix components and thereby leading on to tissue invasion and metastasis.

Recent studies have proved that MMPs play different roles at different stages of tumorogenesis[(22)].Other than tumor angiogenesis, tissue invasion and metastasis, MMPs are also involved in apoptosis of cancer cells. These MMPs

exhibit both pro-apoptotic as well as anti-apoptotic effects[22]. An increased efficacy of MMP inhibitor therapy in cancer treatment is also been extensively studied.



Other than tumor invasion and metastasis, cancer cells are also involved in tumor growth, tumor protection, apoptosis, angiogenesis and metastasis.

1. TUMOR GROWTH

There is an increasing evidence, supporting the participation of MMPs in regulation of tumor growth. The mechanisms involved are:

1. MMPs disrupt the balance between growth and antigrowth signals in the tissue micro-environment[23].

2. MMPs favour the release of cell proliferation factors such as TGF-beta and insulin-like growth factors[23].

3. Direct effect of MMP matrix remodelling activity on cell growth.

   MMPs 1, 2, 7, 9, 11, 13, 14 had a higher expression in cancer cells. Tumors with higher concentrations of MMP-11 and MMP-13 have a significantly greater probability of relapse.

2. TUMOUR PROTECTION

The activity of MMP is associated with a variety of escaping mechanisms that cancer cells develop to avoid host immune responses[22]. For eg; MMP-9, suppresses the proliferation of T lymphocytes through IL-2 signalling disruption. Similarly MMP-11 decreases the sensitivity of tumor cells to natural killer cells. They also modulate antitumor immune reactions by efficiently cleaving several chemokines or regulating their mobilisation. Additionally, they protect the host by stimulating protective and adaptive immune responses.

3. APOPTOSIS

Few MMPs have pro-apoptotic actions, while a few others have anti-apopototic features[22]. MMP-3 and MMP-7 predominantly exhibit pro-apoptotic actions.MMP-11 suppress tumor cell apoptosis inhibiting cancer cell death.

4. ANGIOGENESIS

MMPs are positive regulators of tumor angiogenesis. Many pro-angiogenic factors like vascular endothielial growth factor, basic fibroblast growth factor or transforming growth factor beta are induced or activated by these MMPs[23].They also trigger angiogenic switch during carcinogenesis, thereby facilitating vascular remodelling and neovascularisation at distant sites from a tumor.

The pro-angiogenic effect of MMPs are studied in various cancers. These include B cell chronic lymphocytic leukemia, urothielial carcinomas and ovarian cancers.

5. METASTASIS

MMPs promote metastasis, predominantly by extracellular matrix degradation near the proliferating cells of malignant neoplasm and thereby aid in tumor growth in surrounding tissues.MMP-9 and MMP-14 cleaves collagen type IV and are mainly associated with lung tumors. MMP-2, MMP-9 and MMP-12 cleaves elastin associated microfibrils in various types of tumors.

EFFECTS OF MMPs ON OTHER DISEASES

Elevated levels of MMPs are noted in the synovial membrane, cartilage, tendon and bone of patients with rheumatoid arthritis and osteoarthritis. In both these conditions, inflammatory mediators stimulate the production of MMPs which ultimately degrade most of the extracellular matrix components.MMP-2,MMP-3, MMP-8, MMP-9, MMP-10, MMP-13, MMP-14 and MMP-19 are mainly studied to be elevated in arthritis.

MMPs play a key role in development of various vascular diseases. They are predominantly associated with atherosclerosis. MMPs cause intimal thickening in the walls of large arteries, as a repair response to vessel wall damage. Thereby initiates formation of an atherosclerotic plaque. MMPs are also known to cause atherosclerotic plaque rupture. Studies on association of MMP-14, MMP-2 & MMP-9 in the development of abdominal aortic aneurysms have been proved positive.

Many hereditary diseases are associated with mutations of MMP genes. These mutations results in increased protease activity which leads to marked deficiencies in the turnover of specific extracellular matrix components. One such example is nodulosis-arthropathy-osteolysis (NAO) syndrome.MMP-2 mutations have been reported in three different skeletal disorder, which are collectively called as inherited osteolysis syndrome. This syndrome is characterised by progressive resorption of bones. Similar observations as NAO are also noted in Winchester syndrome and Torg syndrome. MMP-13 gene

mutations are responsible for Missouri type of human spondyloepimetaphyseal dysplasia (SEMD). SEMD is an autosomal dominant disorder and is characterised by defective growth and remodelling of vertebrae and long bones.

An increased role of MMPs in various neurodegenerative disorders like parkinsonism, alzheimers disease, multiple sclerosis and Huntingtons disease are been widely studied these days[24].

INGUINAL HERNIA : LOCAL MANIFESTATION OF A SYSTEMIC DISEASE

Hernia has historically been considered to be caused by mechanical defects in the integrity of abdominal wall. These defects may result from various intrinsic or extrinsic triggering factors. Recently, it is studied that hernia is not just a local mechanical defect but a manifestation of a systemic disease.

There is sufficient evidence to prove that the underlying etiologies of hernia are associated with disturbances in connective tissue metabolism and impaired extracellular matrix turnover.

Collagen is one of the predominant structural protein of transversalis fascia and the main component of extracellular matrix. Type I and Type III collagen are mainly implicated in the formation of inguinal hernia[25].Type I collagen is a highly cross-linked mature collagen and its function is to provide mechanical strength to the fascial layers. On the other hand Type III collagen is

less cross-linked, contains thin fibres and provides less mechanical strength to the tissues.

An imbalance in collagen homeostasis either as a delayed or abnormal collagen synthesis or an increased proteolytic collagen breakdown has been found to have a causal effect on genesis of inguinal hernia[(1)].

An altered collagen Type I : Type III ratio has been extensively demonstrated by several investigators in the fascia of patient with both inguinal as well as incisional hernia. This decrease in collagen Type I: Type III ratio may be due to

1. A primary defect in collagen synthesis

   or

2. Altered collagen degradation due to increased MMP activity.

The increased collagen degradation can be evaluated by measuring the levels of MMP in the fascial layers of the abdomen. Among the various MMPs, MMP-2 has been widely studied in the pathogenesis of inguinal hernia.

Several studies have proved an over expression of MMP-2 in the fibroblasts of both skin and transversalis fascia of inguinal hernia patients compared with their controls[(26)].

An imbalance in the ratio of MMP with its inhibitor TIMP has also been demonstrated in both local tissue level and in systemic circulation of patients with hernia[(27)].Normal MMP:TIMP is equal to 1:1.This alteration in MMP and

TIMP ratio demonstrates a dysregulation of extracellular matrix degradation process.

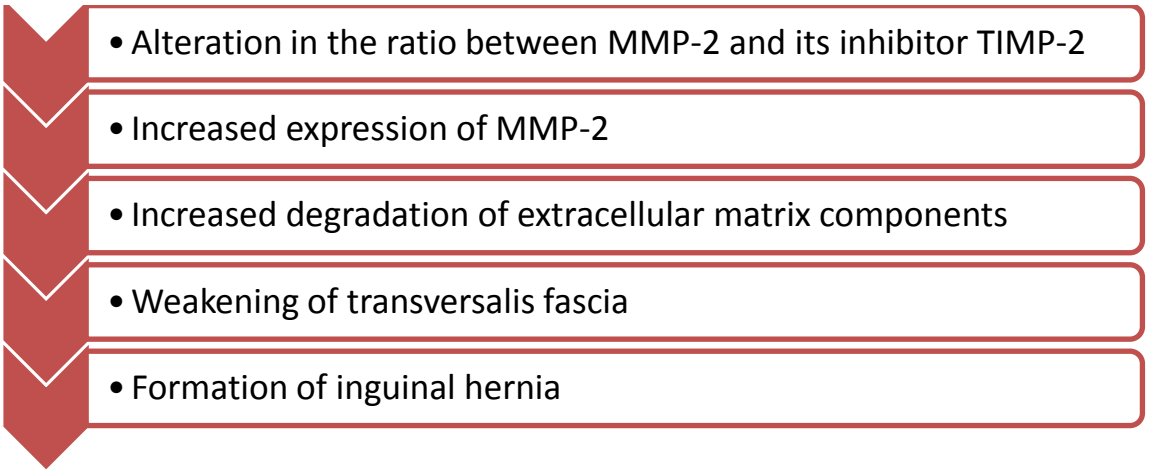
Other than MMP-2, MMP-1 and MMP-13 activity are widely studied and are proved to be implicated in recurrent inguinal and incisional hernia[27].

There is increasing evidence to prove that the defect in connective tissue metabolism is a systemic process. This is supported by the increased serum proteolytic activity in patients with abdominal wall hernia.

Theoritically, highly expressed metalloproteinase at the local tissue level could be continuously released into systemic circulation and can be measured in the plasma of patients with abdominal wall hernias and aneurysms.

ROLE OF MATRIX METALLOPROTEINASE-2 (MMP-2) IN THE PATHOGENESIS OF INGUINAL HERNIA

As already stated matrix metalloproteinases are highly implicated in the genesis of inguinal hernia. Among these, the widely experimented and studied is the role of MMP-2 in the genesis of ingunal hernia.



Various studies on MMP-2 and its relation to inguinal hernia have been conducted. A few amongst them include;

- Matrix Metalloproteinase-2 and its Relation with Incisional & Inguinal Hernia by Jain et al
- Roles of matrix metalloproteinases in the etiology of inguinal hernis by Aren et al.

ASSOCIATION OF ABOMINAL WALL HERNIA WITH ABDOMINAL AORTIC ANEURYSMS

An alteration in collagen type I / collagen type III ratio, which implies an altered collagen metabolism is a common feature in the pathogenesis of abdominal aortic aneurysm, inguinal hernia and incisional hernia[28].

An increased protease activity and an imbalance in protease / antiprotease balance is identified in both abdominal aortic aneurysm and abdominal wall hernia[4].

This increased protease activity can lead on to an abnormal connective tissue remodelling process and thereby causing a disorganised collagen synthesis and degradation. Inflammation is also regarded as an important feature in both these conditions [28].

GENETIC ASPECTS OF ANEURYSM AND HERNIA

Polymorphic gene mutations of various structural components of connective tissue (elastin, collagen), extracellular matrix degrading enzymes

and their inhibitors (MMPs, TIMPs) and inflammation promoting agents have been studied and identified in both hernia and aneurysms[4]. As of now, no single genetic alteration pathognomic of hernias or aneurysms have been identified so far.

Inguinal hernia can be associated with other connective tissue disordes like Ehler-Danlos syndrome and Marfans syndrome. Ehler-Danlos syndrome is associated with type III collagen defect (COL3A1), while Marfans syndrome is related to a mutation in fibrillin -1 gene.

Genetic abnormalities of extracellular matrix components are considered to be associated with a higher risk for development of inguinal hernia. Of these Marfans syndrome and Ehler-Danlos syndrome plays an important role[4].

Other than collagen, elastin, matrix metalloproteinases (MMPs) and their inhibitors, studies regarding the role of other extracellular matrix components in the genesis of abdominal wall hernia and aneurysms are still not been carried out.

To conclude, the ultimate etiology of abdominal wall hernia seems to be underlined by the contribution of genetic, intrinsic patient related and extrinsic environmental factors.

MMP INHIBITOR THERAPY IN INGUINAL HERNIA

Role of MMP inhibitor therapy in inguinal hernia is a highly neglected area of clinical trial. The most commonly used MMP inhibitors are the

tetracyclines. Among the tetracyclines, the most widely used ones are doxycycline and minocycline[29,30]. Of all the tetracyclines, MMP-2 inhibition by doxycycline has been widely studied in the recent past[31].

Other than tetracyclines, chemically modified tetracyclines (CMTs) and synthetic MMP inhibitors are also widely studied. The use of MMP inhibitors are widely experimented in other connective tissue disorders other than inguinal henia, like abdominal aortic aneurysms, cancers, joint disorders, Ehler-Danlos syndrome and Marfans syndrome. Tetracyclines have a broad spectrum of MMP inhibition. These inhibits MMP-1, MMP-2,MMP-7,MMP-8, MMP-9, MMP-11 and MMP-13.

MECHANISM OF ACTION OF TETRACYCLINES

The tetracyclines exhibit many non antibiotic mechanisms also.The most common mechanism of action by which it inhibits MMPs is by binding to the Zn site of MMP enzyme and thereby blocking its action.

Other mechanisms involved are:

- Inhibition of MMPs at the gene expression level.
- Reducing the activation of MMPs via the inflammatory cascade
- Reducing the activation of MMPs via reactive oxygen species.

CHEMICALLY MODIFIED TETRACYCLINES (CMTs)

These have been designed to reduce unnecessary effects on endogenous microbial flora, while retaining the MMP inhibitory action.

The CMTs are now been used in the treatment of chronic periodontitis. Among the various CMTs, CMT-3 is being tested in clinical trials for human cancer[32].

Effect of tetracyclines on endogenous microbial flora can also be avoided by administering a low dose doxycycline. For eg; 20mg twice a day instead of 100mg twice a day.By reducing the dose of doxycycline, its anti MMP activity is retained while its anti-microbial activity is hampered. The commonly used chemically modified tetracyclines include CMT-3/COL-3[31].

The advantages of CMTs over conventional tetracyclines include their rapid absorption, longer half life and absence of development of antibiotic resistant microbial flora[32].

SYNTHETIC MMP INHIBITORS

These synthetic inhibitors represent a heterogenous grouo of compounds. They have an increased inhibitory potency and a high specificity against various MMPs. The commonly experimented synthetic MMP inhibitors include,GM6001, BB-1101[33].

OTHER MMP INHIBITORS

The bone resorption inhibitors namely bisphosphonates exhibit a high MMP inhibitory activity. They act via the mechanism of cation chelation[34, 35]. The other synthetic MMP inhibitors, that are experimentally studied for various connective tissue disorders include:

- Succinyl hydroxamates
- Sulphonamide hydroxamates
- Phosphinamide hydroxamates
- Carboxylate inhibitors
- Thiol inhibitors
- Aminomethyl benzimidazole analogue
- Peptides

Majority of these MMP inhibitors are studied and utilised against MMP-2, as this protease is involved in the pathophysiology of multiple disorders including tumor invasion and metastasis, rheumatoid arthritis, hernia etc[35].

The experiments on the effect of MMP inhibitors are predominantly conducted on various connective tissue disorders including rheumatoid arthritis, various cancers, systemic sclerosis, abdominal aneurysms, abdominal dissections and various other joint disorders[36,37].

However, experimental studies on the role of MMP inhibitors in ingiuinal hernia is still been neglected[38]. Among the various MMP inhibitors, doxycycline has been proved to inhibit MMP-2 and MMP-9[39,40] large population-based prospective study to explore the feasibility of the use of MMP inhibitors for hernia treatment is highly recommended.

MATERIALS AND METHODOLOGY

This is a prospective case control study. The study period was from November 2015 to January 2017. Those patients admitted with non recurrent inguinal hernia and undergoing laproscopic or open hernioplasty were included in our study. The exclusion criterion were the following:

- Age less than 18yrs and more than 75 yrs
- Patients on steroid medication
- Smokers
- Patients who had undergone previous infraumbilical abdominal procedures
- Recurrent hernia patients
- Patients with known connective tissue disorders
- Non consenting patients

The study population was divided into two groups:

1. Group A (Hernia /Case group); This group included patients with non recurrent inguinal hernia (unilateral /bilateral)
2. Group B (Control group); This included patients undergoing intra-abdominal procedures (laprotomy /laproscopy for causes other than any hernia of the abdominal wall such as femoral hernia, umbilical or paraumbilical hernia and incisional hernia)

Sample size in each group:

Group A ; 20

Group B; 20

Biopsy specimens of skin and transversalis fascia from 20 patients of hernia group and 20 patients of control group were received in the pathology laboratory of PSGIMSR. These biopsies which was received in our laboratory were allotted a specific reference number, which aids in their later identification from the archives.

These specimens were processed and submitted for hematoxylin and eosin (H&E) stain. To confirm the presence of collagen bundles, a special stain called Massons trichrome was also used.

As our study included the use of antibodies to detect MMP-2 antigens, the tissues were also processed separately in Poly- L- Lysine coated slides.

The slides stained with hematoxylin and eosin were used for routine histological examination, while the Massons trichrome stain was used to confirm the presence of collagen fibres.

The slides coated with Poly-L-Lysine, underwent immunohisto-chemistry staining for detection of MMP-2 in the fibroblasts of skin and transversalis fascia.

These were scored as follows:

1+; 0-25% of fibroblasts show MMP-2 staining

2+; 25-50% of fibroblasts show MMP-2 staining

3+; 50-75% of fibroblasts show MMP-2 staining

4+; 75-100% of fibroblasts show MMP-2 staining

HEMATOXYLIN AND EOSIN STAIN

The hematoxylin and eosin(H&E) is the most widely used stain in a histopathology laboratory. The advantage of H&E lies in its simplicity and ability to clearly demonstrate enormous number of different tissue structures. The hematoxylin component stain the nuclei blue, while the eosin component stains the cytoplasm and most of the connective tissue fibres.

There are various methods of performing the H&E staining, we commonly use the Harris Hematoxylin method. This is a type of alum hematoxylin. It is chemically oxidised with mercuric oxide. As mercuric oxide is toxic, we commonly use sodium or potassium iodate as a substrate for oxidation.

Materials required includes:

- Harris hematoxylin
- Eosin
- Xylene

- 1% acetic acid
- Ammonia water
- 1% eosin
- Graded alcohols

PROCEDURE

	Deparaffinse the sections
	Hydrate through graded alcohols
	Stain in Harris hematoxylin for 5 minutes
	Wash in running tap water until the sections turn blue
	Differentiate in 1% alcohol for 5-10 seconds
	Blueing with ammonia water
	Counterstaining in eosin for 1minute
	Wash in tap water
	Dehydrate through alcohols
	Clear and mount

The staining was done in routine paraffin sections.

RESULTS:

Nuclei ; blue

Cytoplasm ; pink

Red cells; orange

Fibrin ; deep pink

2. MASSONS TRICHROME STAIN

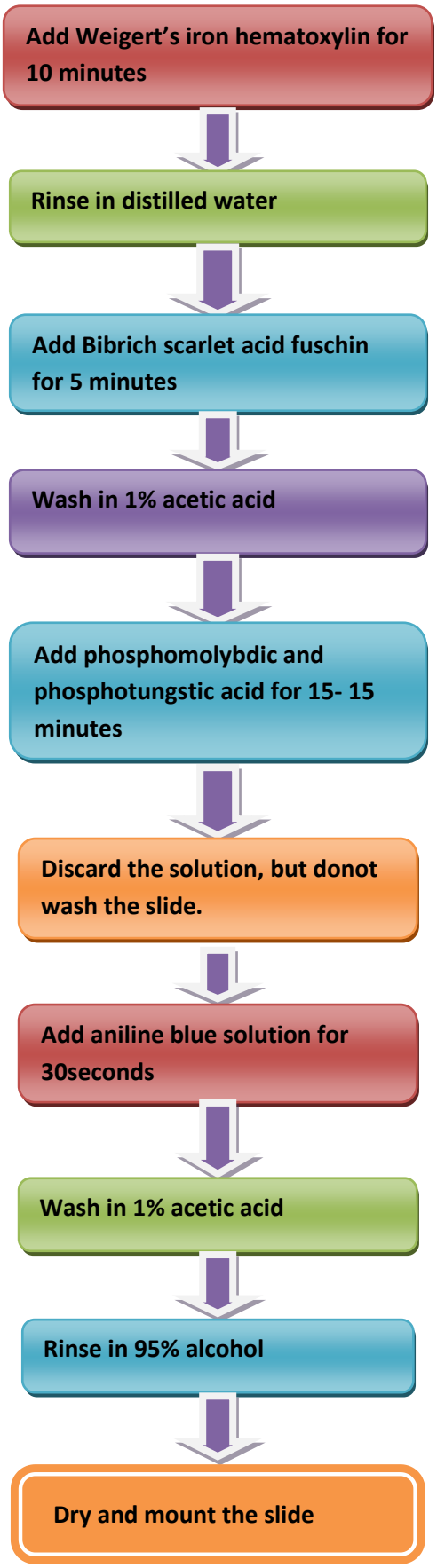
These belong to the category of trichrome stains, which are used for staining various connective tissues. This technique is mainly used for selective demonstration of muscle, collagen fibres, fibrin and erythrocytes. As the name suggests it is a combination of three stains of which one may be a nuclear stain.

MATERIALS USED

1. Weigert's iron hematoxylin solution
2. Bibrich scarlet- acid fuschin
3. Phosphomolybdic phosphotungstic acid solution
4. Aniline blue solution
5. 1% glacial acetic acid

Nuclear stain is by Weigert's iron hematoxylin. Phosphomolybdic acid and phosphotungstic acid, both of them acts as mordants.

PROCEDURE:


Add Weigert's iron hematoxylin for 10 minutes
Rinse in distilled water
Add Bibrich scarlet acid fuschin for 5 minutes
Wash in 1% acetic acid
Add phosphomolybdic and phosphotungstic acid for 15- 15 minutes
Discard the solution, but donot wash the slide.
Add aniline blue solution for 30seconds
Wash in 1% acetic acid
Rinse in 95% alcohol
Dry and mount the slide

RESULT

1. Collagen; blue

2. Muscle and fibrin; red

3. Nuclei; blue black

3. STAINING FOR ANTIBODY

The sections are cut at a thickness of about 4 micrometer. These are then floated on to Poly-L - Lysine coated slides and incubated at 37 degree for one day and further incubated at 58 degree overnight.

PRECAUTIONS TO BE TAKEN

- The sections are not allowed to dry at any stage of the procedure.
- The steps of incubation with antibody are carried out at a temperature of 37 degrees.
- Adequate controls for each antibody tested are to be used.

PROCEDURE

i) DEPARAFFINIZATION

Xylene – I - 15 minutes

Xylene- II - 15 minutes

ii) DEXYLENIZATION

Absolute Alcohol- I - 1 minute

Absolute Alcohol - II - 1 minute

iii) DEALCOHOLISATION

90% Alcohol - 1 minute

70% Alcohol - 1 minute

iv) REHYDRATION

Rinse in tap water for 10 minutes

v) Rinse in distilled water for 5 minutes.

vi) HEAT INDUCED ANTIGEN RETRIEVAL

Pressure cooked in citrate buffer at a ph-6 for 10 minutes.

vii) Leave the pressure cooker in a sink with water for 20 minutes and is cooled to room temperature.

viii) Rinse in distilled water for 5minutes.

ix) Transfer into trisodium phosphate buffer (Ph 7.6) for 5 minutes each for two times.

x) Peroxidase block for 10- 15 minutes. In this step 3% hydrogen peroxide in distilled water is used inorder to prevent nonspecific background staining.

xi) Wash in TBS buffer for 5 minutes each for three times.

xii) Power block for 15 minutes. This is to block non specific reaction with other tissue antigens. The blocking reagent used is casein in PBS with 15MM sodium azide.

xiii) ADDITION OF PRIMARY ANTIBODY;

Drain and cover the section with specific marker and wait for 1 hour for the antigen antibody reaction to take place.

xiv) Wash in TBS buffer , 5 minutes each for 3 times. This is to remove any unbound antibodies.

xv) Super enhancer for 30 minutes. This is to enhance the reaction between primary and secondary antibody.

xvi) Wash in TBS buffer, 5 minutes each for three times. This is to remove any unbound antibodies.

xvii) Addition of super sensitive poly – HRP for 30 minutes.

xviii) Wash in TBS buffer, 5 minutes each for three times. This step is to remove any unbound antibodies.

xix) Addition of a colour development solution or chromogen (3, 3 Diamino Benzidine tetra hydrochloride-DAB) for 5-8 minutes. This acts as a chromogen and imparts colour.

xx) Wash in TBS buffer, 5 minutes each for three times.

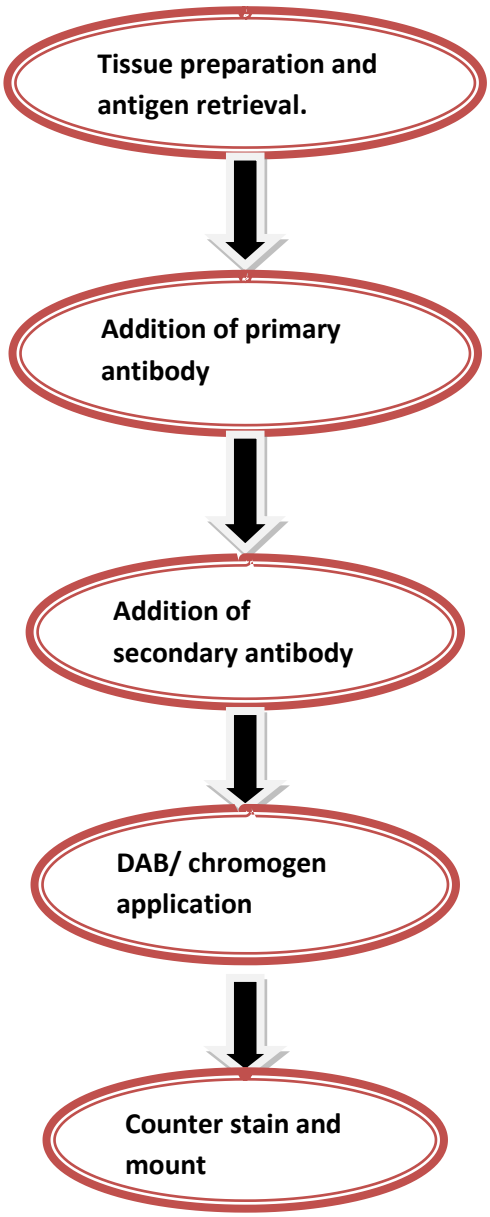
xxi) Wash in tap water for 5 minutes

xxii) Counterstain with Harris hematoxylin for 1 minute

xxiii) Wash in tap water for 5 minutes to remove excess of stain

xiv) Air dry, clear in xylene and mount with DPX.

To summarise, the steps of antibody staining includes the following;



RESULT

- Fibroblast of skin and transversalis fascia shows a cytoplasmic positivity for MMP-2
- Internal controls are basal cells of epidermis and endothielial cells.

Fibroblasts, basal cells of epidermis and endothielial cells show a brown coloured cytoplasmic positivity for MMP-2.

The clone of MMP-2 used in our study is;

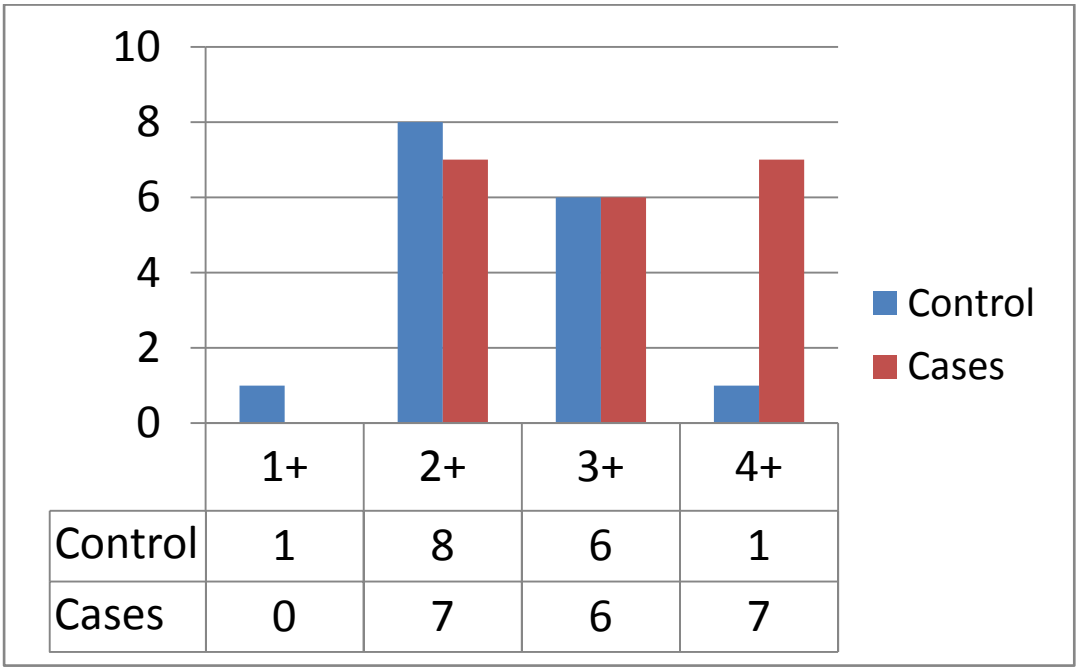
IM51-100UGCN Anti-MMP-2 (Ab-4) Mouse mAb (75-7F7)-- 100 UG

RESULTS

Department of pathology, for the study purpose received 20 biopsy specimens of skin and transversalis fascia of patients with inguinal hernia . Also received were 20 biopsy specimens from skin and transversalis fascia of matched control population. A complete histopathological examination with H&E followed by comparison of MMP-2 immunohistochemical staining pattern among the hernia cases and controls was carried out. Within the result, we have included the following:

- A general comparison of MMP-2 expression among the skin and transversalis fascia of cases and controls.
- Percentage analysis of MMP-2 scoring pattern in the skin and transversalis fascia of cases and controls.
- Percentage analysis of MMP-2 scoring pattern in the skin and transversalis fascia of patients with direct and indirect inguinal hernia.
- Age-wise distribution of MMP-2 scoring in skin and transversalis fascia of cases and controls.

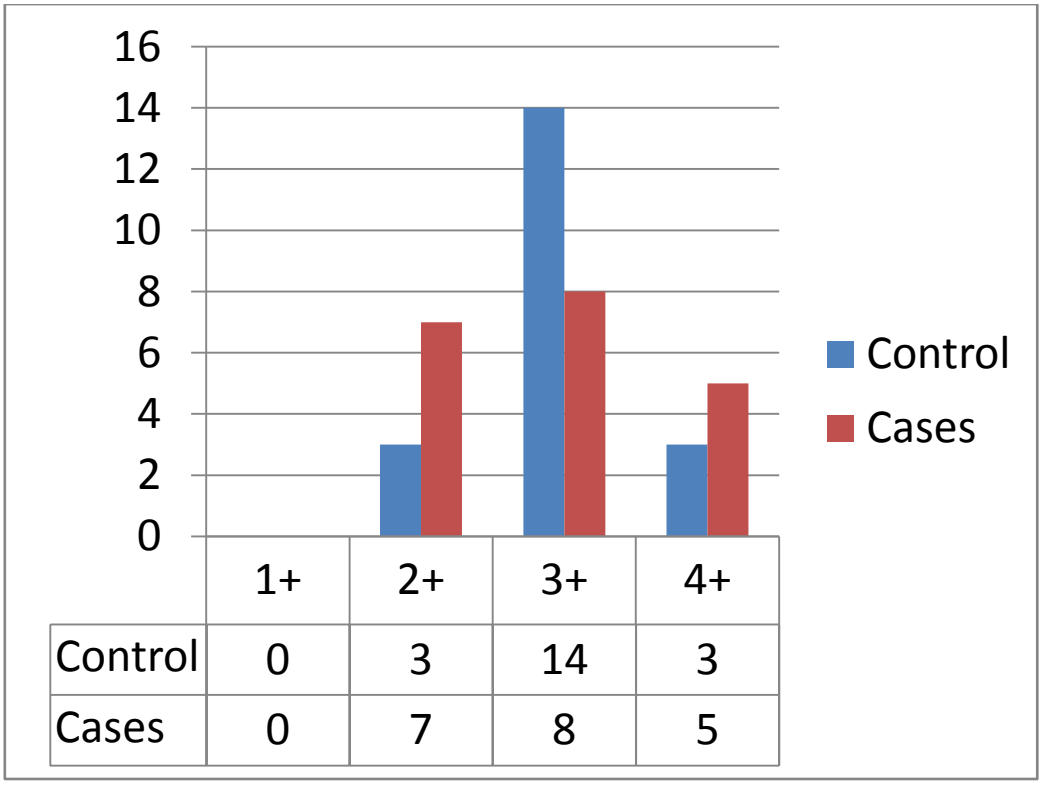
The results are as follows:



	1+	2+	3+	4+
Control	1	8	6	1
Cases	0	7	6	7

FIG:2 Compares the MMP-2 expression among skin of cases and controls.

Among a total number of 20 inguinal hernia cases, seven of them showed a 4+ MMP-2 scoring pattern in the fibroblasts of skin, while only 1 out of 20 controls showed a similar MMP-2 scoring. A 3+ scoring was found in an equal number in both cases as well as control population.



	1+	2+	3+	4+
Control	0	3	14	3
Cases	0	7	8	5

FIG:3 Compares the MMP-2 expression among transversalis fascia of cases and controls

Among the 20 cases studied, a 4+ MMP-2 scoring in the fibroblast of transversalis fascia was expressed in 5 patients with inguinal hernia. While a similar MMP-2 scoring pattern was expressed by 3 out of 20 among the control population. A 3+ scoring pattern was expressed at a higher rate among the control population, the explanation for this is been discussed later.

MMP-2	CONTROL		CASES	
	No of Cases	%	No of Cases	%
1+	1	5%	0	0%
2+	8	60%	7	35%
3+	6	30%	6	30%
4+	1	5%	7	35%

TABLE:8 Percentage analysis of MMP-2 scoring pattern in the skin of controls and cases

About 35% of cases with inguinal hernia shows a 4+ MMP-2 scoring pattern in the fibroblasts of skin, while only 5% of the control population shows a similar MMP-2 expression.

MMP-2	CONTROL		CASES	
	No of Cases	%	No of Cases	%
1+	0	0%	0	0%
2+	3	15%	7	35%
3+	14	70%	8	40%
4+	3	15%	5	25%

TABLE:9 Percentage analysis of MMP-2 scoring pattern in the transversalis fascia of controls and cases.

In TABLE 9; a 4+ MMP-2 scoring pattern is expressed by 25% of cases, while 15% of controls shows a similar MMP-2 expression in the fibroblasts of transversalis fascia.

MMP-2	DIRECT INGUINAL HERNIA		IINDIRECT INGUINAL HERNIA	
	No of Cases	%	No of Cases	%
1+	0	0%	0	0%
2+	3	30%	4	40%
3+	3	30%	3	30%
4+	4	40%	3	30%

TABLE:10 Percentage analysis of MMP-2 scoring pattern in the skin of patients with direct and indirect inguinal hernia

MMP-2	DIRECT INGUINAL HERNIA		IINDIRECT INGUINAL HERNIA	
	No of Cases	%	No of Cases	%
1+	0	0%	0	0%
2+	3	30%	3	30%
3+	4	40%	4	40%
4+	3	30%	3	30%

TABLE:11 Percentage analysis of MMP-2 scoring pattern in the transversalis fascia of patients with direct and indirect inguinal hernia

Table 10& 11 depicts a percentage analysis in the MMP-2 scoring pattern in the skin and transversalis fascia of patients with direct and indirect inguinal hernia. Our study, shows a similar MMP-2 activity in both direct and indirect inguinal hernia patients.

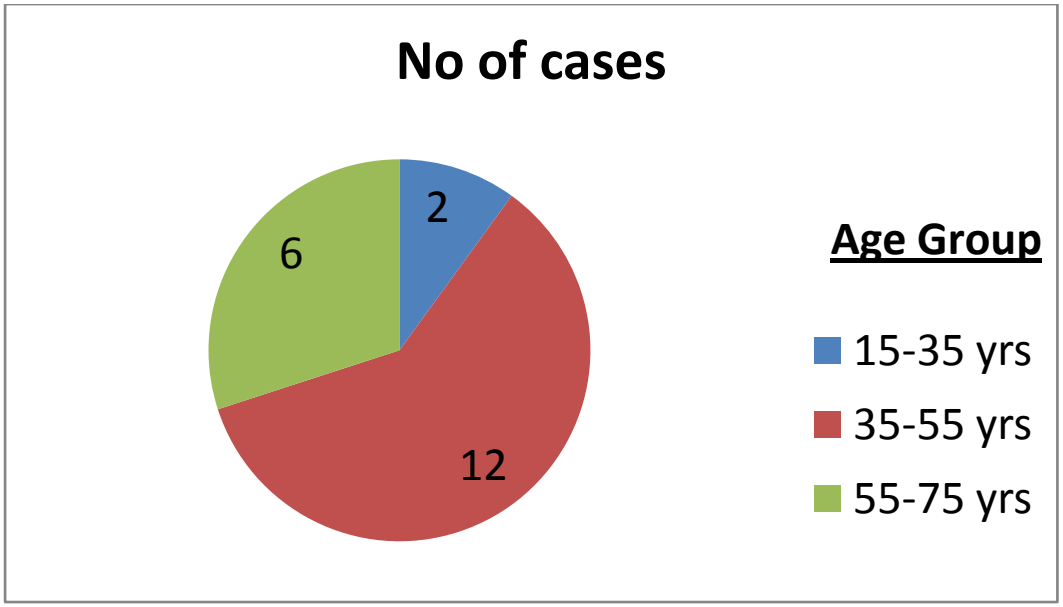


FIG.4 : AGE-WISE DISTRIBUTION OF CASES

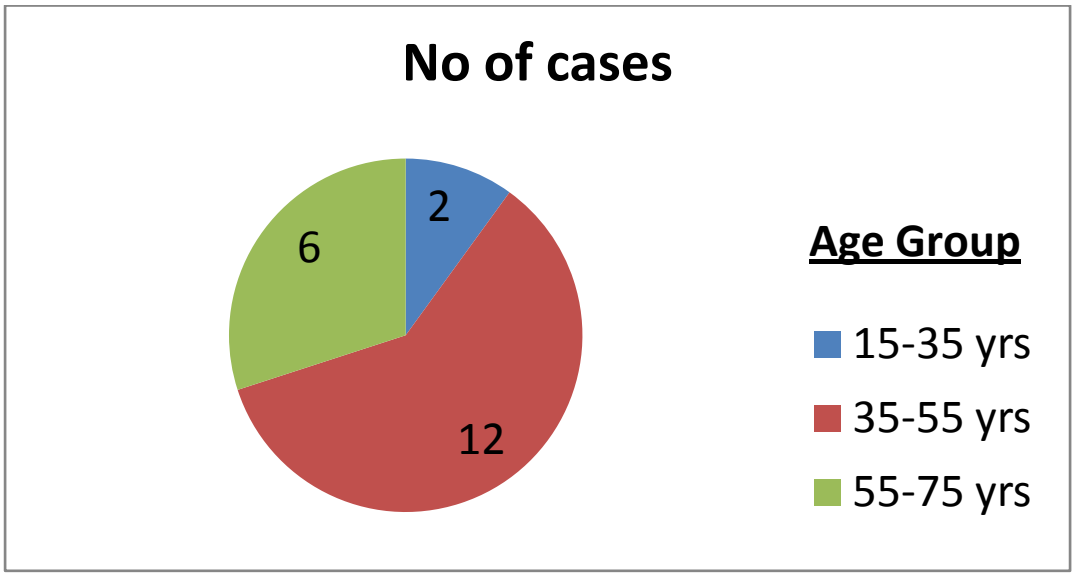


FIG.5 : AGE-WISE DISTRIBUTION OF CONTROLS;

AGEWISE COMPARISON OF MMP-2 EXPRESSION IN HERNIA PATIENTS AND THEIR CONTROLS

2 cases and controls in the age group of 15-35 yrs, 12 cases and controls in the age group of 35-55 yrs and 6 cases and controls in the age group of 55-75yrs were analysed in our study.

AGE GROUP	TOTAL NO OF CASES	MMP SCORE							
		1+		2+		3+		4+	
		No of cases	%	No of cases	%	No of cases	%	No of cases	%
15-35 yrs	2	0	0%	2	100%	0	0%	0	0%
35-55 yrs	12	0	0%	3	25%	4	33.33%	5	41.60%
55-75 yrs	6	0	0%	2	33.33%	2	33.33%	2	33.33%

TABLE:12 Age-wise distribution of MMP-2 scoring pattern in the skin of hernia cases

A 4+ scoring pattern of MMP-2 in the fibroblasts of skin is expressed by 41.60% of hernia patients in the age group of 35-45yrs. Among the age group of 55-75yrs about 33.33% of hernia patients express a 4+ scoring pattern of MMP-2 in their skin fibroblast as depicted in table 12.

AGE GROUP	TOTAL NO OF CONTROLS	MMP SCORE							
		1+		2+		3+		4+	
		No of cases	%	No of cases	%	No of cases	%	No of cases	%
15-35 yrs	2	0	0%	0	0%	2	100%	0	0%
35-55 yrs	12	0	0%	9	75%	3	25%	0	0.00%
55-75 yrs	6	1	16.66%	3	50%	1	16.66%	1	16.66%

TABLE:13 Age-wise distribution of MMP-2 scoring pattern in the skin of control group

In the age group of 15-35yrs,100% of the controls shows a 2+ MMP-2 scoring their skin fibroblast. Among the age group of 35-55yrs about 75% of the controls show a 2+ MMP-2 scoring in their skin fibroblast. In the age group of 55-75yrs, about 16.66% each of 1+, 2+, 3+, and 4+ scoring of MMP-2 in the skin fibroblasts is noted as depicted in table 13.

AGE GROUP	TOTAL NO OF CASES	MMP SCORE							
		1+		2+		3+		4+	
		No of cases	%	No of cases	%	No of cases	%	No of cases	%
15-35 yrs	2	0	0%	0	0%	1	50%	1	50%
35-55 yrs	12	0	0%	3	25%	6	50%	3	25%
55-75 yrs	6	0	0%	4	66.66%	1	16.66%	1	16.66%

TABLE:14 Age-wise distribution of MMP-2 scoring pattern in the transversalis fascia of inguinal hernia cases

AGE GROUP	TOTAL NO OF CASES	MMP SCORE							
		1+		2+		3+		4+	
		No of cases	%	No of cases	%	No of cases	%	No of cases	%
15-35 yrs	2	0	0%	1	50%	1	50%	0	0%
35-55 yrs	12	0	0%	7	58.33%	5	41.66%	0	0.00%
55-75 yrs	6	0	0%	4	66.66%	2	33.33%	0	0.00%

TABLE:15 Age-wise distribution of MMP-2 scoring pattern in the transversalis fascia of control group

Table 14 and 15 depicts the age wise distribution of MMP-2 scoring in the transversalis fascia of cases and controls. In the age group of 15-35yrs about 50% each of 3+ and 4+ MMP-2 scoring is expressed among the cases. While in the control population, 50% each of 2+ and 3+ MMP-2 scoring is expressed. None of the control group shows a 4+ MMP-2 score in this age group.

In the age group of 35-55yrs, an MMP-2 scoring of 4+ is expressed by 25% of the cases and a 3+ scoring is expressed by 50% of the cases with inguinal hernia. Among the control population, about 41.66% express a 3+ MMP-2 score and 58.33% express a 2+ MMP-2 score. A 4+ scoring pattern is not expressed on the control population of this age group.

In the age group of 55-75yrs, about 66.66% of the cases express a 2+ MMP-2 scoring, while 16.66% each of 3+ and 4+ scoring pattern is observed. Among the control population, about 66.66% express a 2+ MMP-2 score, while about 33.33% of these controls shows a 3+ scoring pattern. A 4+ scoring pattern is not expressed in the controls of this age group.

DISCUSSION

The exact etiology of inguinal hernias is still being widely experimented and remains unclear. Many factors predisposing to hernias have been proposed which includes; an open processus vaginalis, increased intraabdominal pressure, impaired sphincter mechanism, familial predisposition, malnutrition, iatrogenic factors, impaired collagen metabolism and increased secretion of matrix metalloproteinases (MMPs)[41,42]. Our study focussed on the role of MMP-2 in the pathophysiology of inguinal hernias.

The degradation of various extracellular matrix components predominantly collagen is controlled by an equilibrium between matrix metalloproteinases and their inhibitors[43].Matrix metalloproteinases (MMPs) comprise a family of zinc endopeptidases, which causes both physiological as well as pathological destruction of the various connective tissue components. Their optimal enzymatic activity is exhibited at a neutral PH[44]. More than twenty different proteins belonging to the family of matrix metalloproteinases are being identified, but the DNA sequences of only eight of these peptidases are identified so far.

In many recent studies, inguinal hernias have been described as occurring due to a defect in collagen metabolism[45, 46]. An alteration in the ratio of collagen I / collagen III is widely being experimented. One such study was conducted by Amal et al, from the Al-Azhar university of Egypt. The study concluded that there is a substitution of collgen type I by collagen type III. This

reduces the tensile strength and mechanical stability of the transversalis fascia, which is of significant importance in the pathophysiology of inguinal hernias.

An excessive degradation of collagen in the transversalis fascia of patients with inguinal hernia is noted[26]. Understanding the role of protease-antiprotease imbalance in inguinal hernia has now shed some light on the reasons for failure of hernia repairs. A high recurrence rate observed in elderly patients following hernia repairs, is related to fibroconnective tissue weakness and impaired collagen metabolism Increased physical activity can be considered only a triggering or secondary cause in the development of inguinal hernias.

An age matched comparison of MMP-2 expression in inguinal hernia patients and their controls were made in our study as depicted in tables 12, 13, 14 and 15.

In the age group of 15-35 years, 50% of hernia patients showed a high(4+) MMP-2 score in the fibroblasts of transversalis fascia as shown in figure 18.A similar MMP-2 expression was not found among the control population. From the above findings, we could infer that a significant increase in MMP-2 expression is noted in the hernia patients compared to their controls. These findings are similar to a study conducted by J. Smigielski et al in 2007, which showed a significant increase in tissue and serum MMP-2 levels of hernia patients compared to their controls.These findings, thereby implicates a predominant role of MMP-2 in the pathophysiology of inguinal hernia.

Among the age group of 35-55years, a high MMP-2 score(4+) was expressed by 41.66% of hernia cases in the fibroblasts of transversalis fascia as shown in figure 18, while none of the controls showed a similar MMP-2 expression .Therefore the findings in this age group were almost similar to that obtained in the age group of 15-35 years, which further highlights on the increased expression of MMP-2 in inguinal hernia patients.

In the age group of 55-75 years, a higher(4+) score of MMP-2 expression in the skin fibroblasts were found in 33.33% of hernia patients and 16.66% of control population as shown in figures 13 and 14 . This mild increase in MMP-2 scoring in the control population could be attributed to the presence of patients with acute appendicitis and malignancy, all of which are associated with an increased expression of MMP-2 [47,48].

A comparison, on the expression of MMP-2 among direct and indirect inguinal hernia patients were also analysed in our study. The study concluded a similar MMP-2 expression pattern in both direct and indirect inguinal hernias as depicted in table 10 and 11. A study by Ziemniak et al in 2009, have reported that the tissue and serum MMP-2 levels were higher in patients with direct and recurrent inguinal hernias than in patients with primary indirect inguinal hernia[49]. Even though a specific role of MMP-2 in the formation of indirect inguinal hernia could not be found out in the current literature, MMP-2 levels are significantly higher in both direct and indirect inguinal hernia groups compared to their controls in our study. A study by Rosch et al, proved that MMP-2 enzymatic activity was similar in patients with recurrent incisional

hernia as compared to their controls[50]. However, in our study patients with incisional hernias are excluded.

CONCLUSION AND SUMMARY

- Our study was a prospective case control study, which included 20 cases of inguinal hernia and 20 controls.
- Inguinal hernia patients showed a considerable increase in the expression of MMP-2 in the fibroblasts of skin and transversalis fascia as compared to their control group.
- This proves the hypothesis that inguinal hernia is a local manifestation of a systemic disease rather than a mere local mechanical defect and also emphasises on the role of MMP-2 in the pathophysiology of hernias.
- An increase in MMP-2 expression was also noted in the controls with malignancy and acute appendicitis, both of which conditions are proved to have an elevated MMP-2 levels.

However, a larger population based study is required to emphasize on the pathophysiological role of MMP-2 in inguinal hernia.

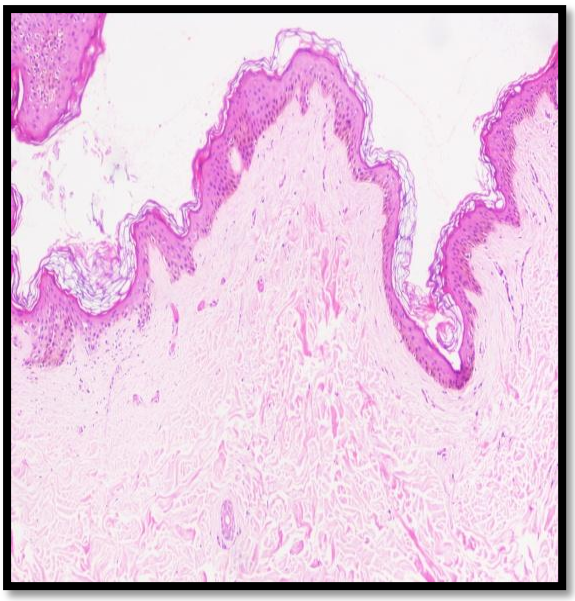


FIG 6: H&E SECTION OF SKIN

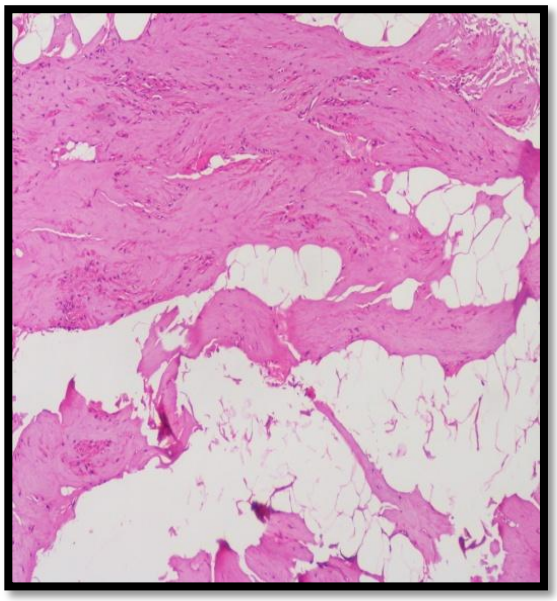


FIG 7: H&E SECTION OF TRANSVERSALIS FASCIA

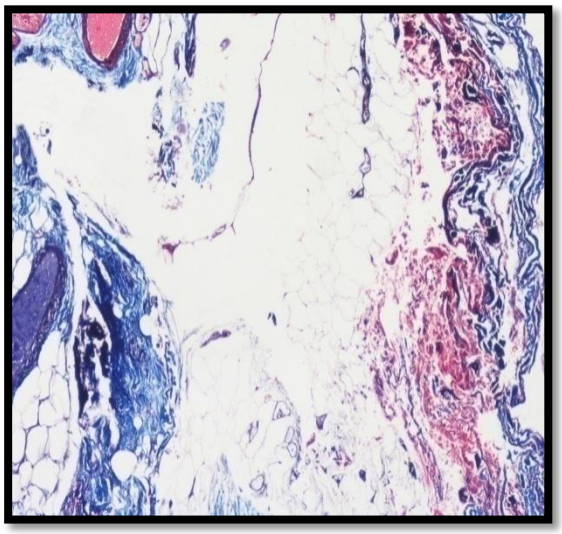


FIG 8: MTS STAIN TO HIGHLIGHT COLLAGEN

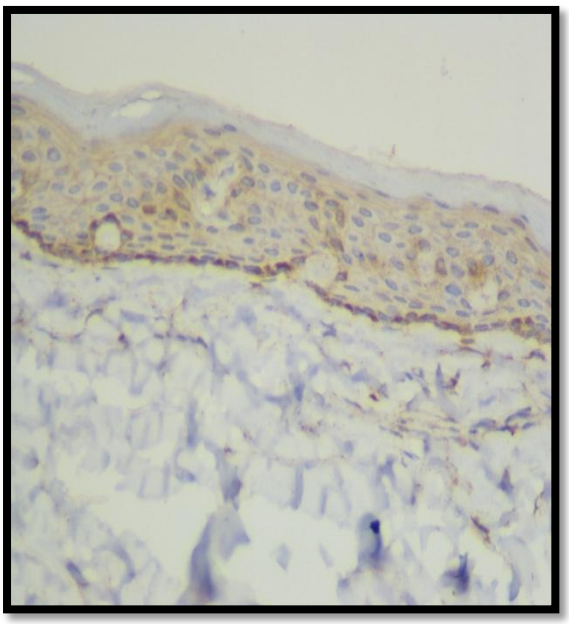


FIG 9: 2+ MMP-2 SCORE IN THE FIBROBLASTS OF SKIN IN HERNIA

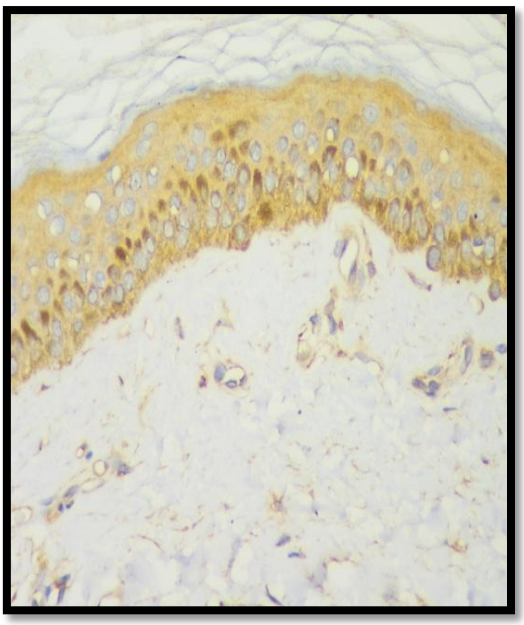


FIG 10: 2+ MMP-2 SCORE IN THE FIBROBLASTS OF SKIN IN CONTROL

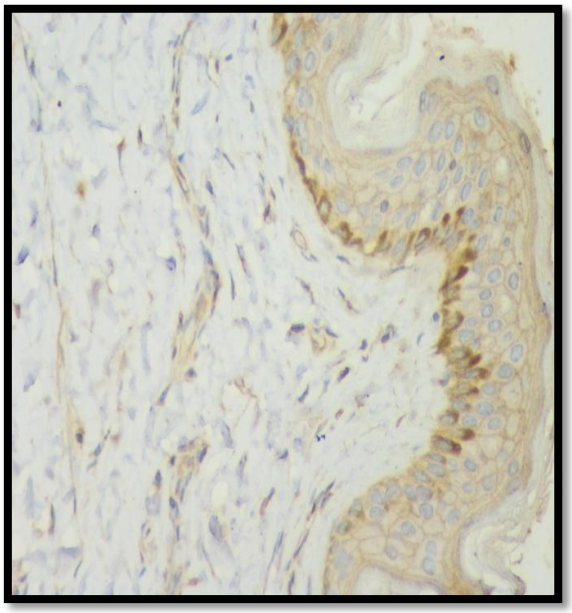


FIG 11: 3+ MMP-2 SCORE IN THE FIBROBLASTS OF SKIN IN HERNIA

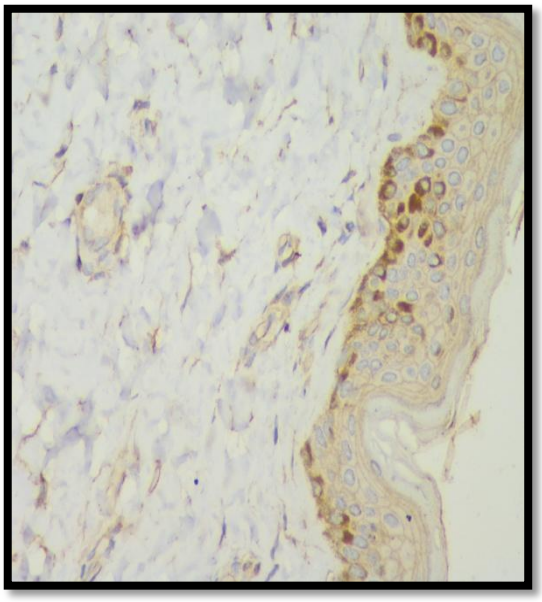


FIG 12: 3+ MMP-2 SCORE IN THE FIBROBLASTS OF SKIN IN CONTROLS

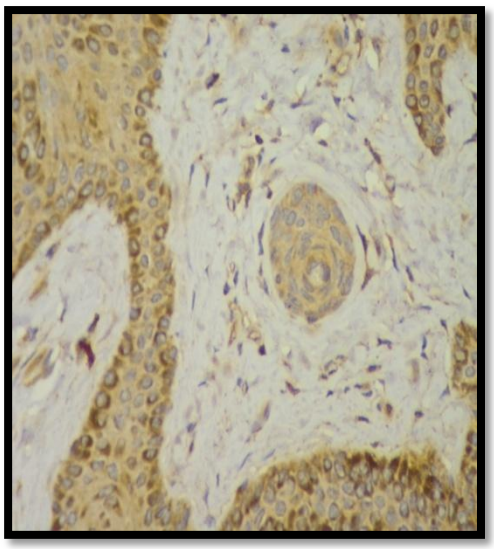


FIG 13 : 4+ MMP-2 SCORE IN THE FIBROBLASTS OF SKIN IN HERNIA CASES

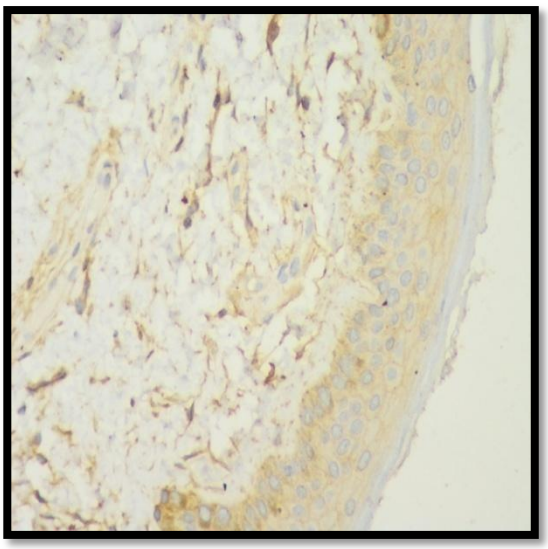


FIG 14: 4+ MMP-2 SCORE IN THE FIBROBLASTS OF SKIN IN CONTROL

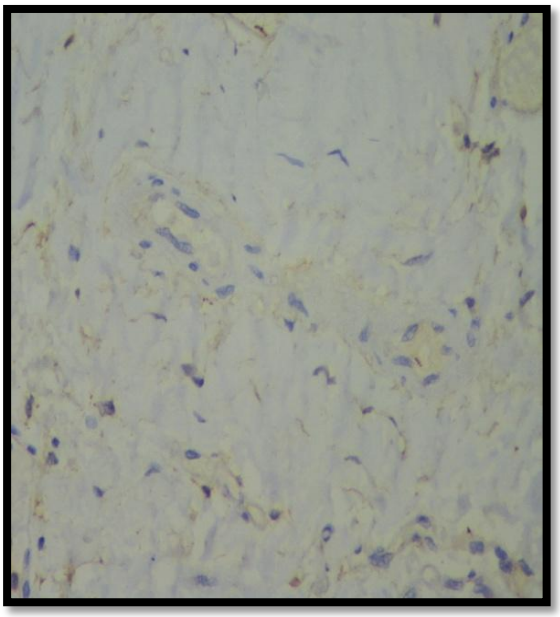


FIG 15: 2+ MMP-2 SCORE IN THE FIBROBLAST OF TRANSVERSALIS FASCIA IN HERNIA CASES.

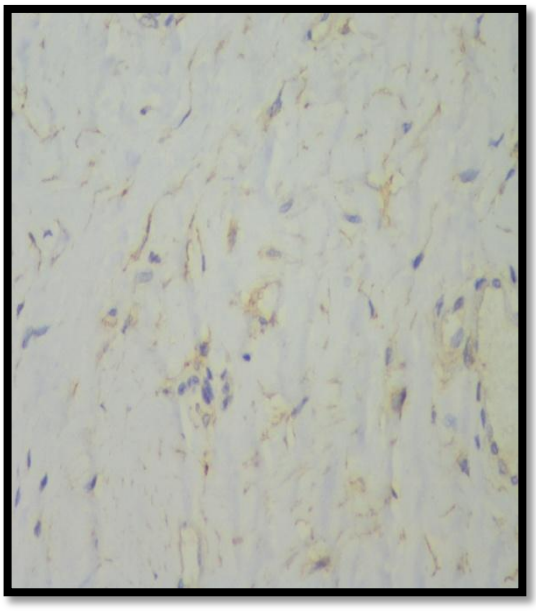


FIG 16: 2+ MMP-2 SCORE IN THE FIBROBLAST OF TRANSVERSALIS FASCIA IN CONTROL

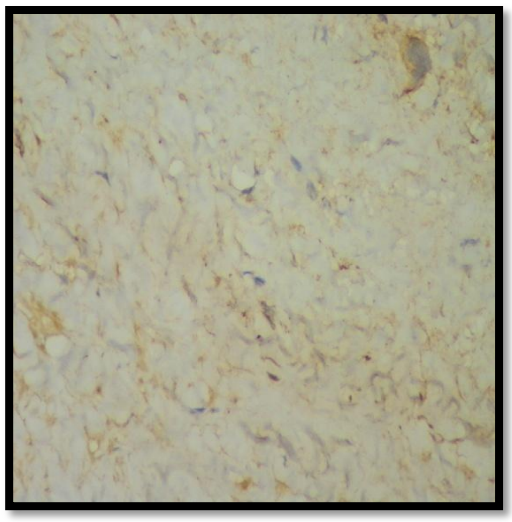


FIG 17: 3+ MMP-2 SCORE IN THE FIBROBLAST OF TRANSVERSALIS FASCIA IN HERNIA CASES

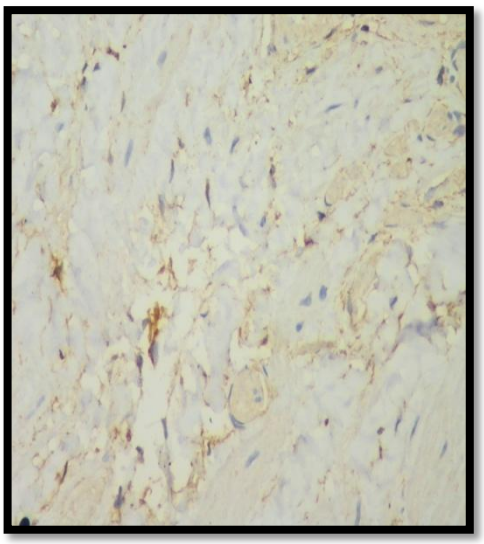


FIG 18: 3+ MMP-2 SCORE IN THE FIBROBLASTS OF TRANSVERSALIS FASCIA IN CONTROLS

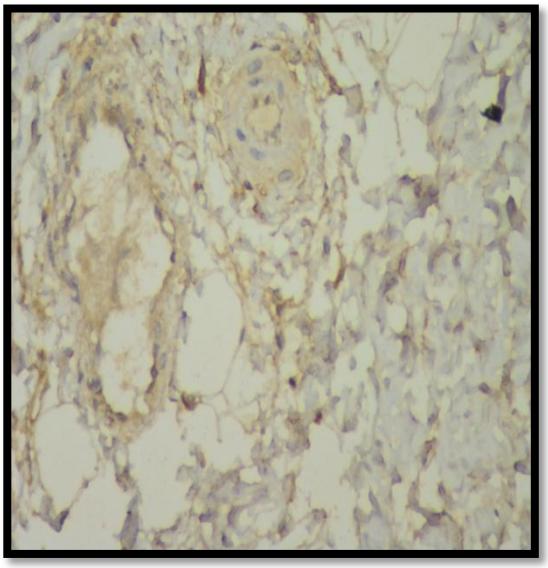


FIG 19: 4+ MMP-2 SCORE IN THE FIBROBLAST OF TRANSVERSALIS FASCIA IN HERNIA CASES

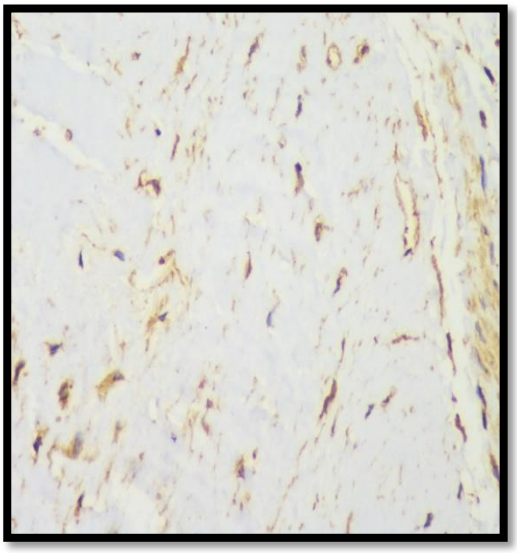


FIG 20: 4+ MMP-2 SCORE IN THE FIBROBLAST OF TRANSVERSALIS FASCIA IN CONTROL

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MMP-2 SCORING IN FIBROBLAST OF SKIN AND TRANSVERSALIS FASCIA (TF) IN HERNIA PATIENTS

SL. NO	AGE	SEX	CLINICAL DIAGNOSIS	HISTOPATHOLOGY DIAGNOSIS		MMP 2 SCORE	
				SKIN	TF	SKIN	TF
1	50	M	INDIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	3+	3+
2	62	M	LEFT INDIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	4+	2+
3	37	M	B/L DIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	4+	3+
4	36	M	RT DIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	3+	3+
5	19	M	LEFT INDIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	4+
6	59	M	B/L DIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	2+
7	50	M	RT DIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	4+	2+
8	53	M	LEFT INDIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	4+	3+
9	38	M	LEFT DIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	3+	2+
10	51	M	RT DIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	4+	2+
11	39	M	RT INDIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	3+	4+
12	52	M	B/L DIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	4+
13	49	M	RT INDIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	3+
14	51	M	B/L DIRECT INGUINAL HERNIA	NO SPECIFIC PATHOLOGY	NO SPECIFIC PATHOLOGY	2+	3+
15	58	M	RT DIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	4+	3+
16	56	M	RT INDIRECT INGUINAL HERNIA	NO SPECIFIC PATHOLOGY	NO SPECIFIC PATHOLOGY	3+	2+
17	60	M	LEFT INDIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	NO SPECIFIC PATHOLOGY	2+	2+
18	62	M	RT DIRECT INGUINAL HERNIA	NO SPECIFIC PATHOLOGY	NO SPECIFIC PATHOLOGY	3+	4+
19	29	M	RT DIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	NO SPECIFIC PATHOLOGY	2+	3+
20	51	M	LEFT INDIRECT INGUINAL HERNIA	MILD CHRONIC INFLAMMATION	NO SPECIFIC PATHOLOGY	4+	4+

MMP-2 SCORING IN FIBROBLAST OF SKIN AND TRANSVERSALIS FASCIA (TF) IN CONTROL

SL. NO	AGE	SEX	CLINICAL DIAGNOSIS	HISTOPATHOLOGY DIAGNOSIS		MMP 2 SCORE	
				SKIN	TF	SKIN	TF
1	47	F	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	3+	2+
2	41	M	VARICOCELE	MILD CHRONIC INFLAMMATION	NO SPECIFIC PATHOLOGY	3+	3+
3	47	M	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	3+
4	42	F	HYPERSLEENISM	NO SPECIFIC PATHOLOGY	NO SPECIFIC PATHOLOGY	2+	3+
5	69	M	ACUTE APPENDICITIS	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	3+	2+
6	45	M	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	2+
7	50	F	VARICOCELE	NO SPECIFIC PATHOLOGY	NO SPECIFIC PATHOLOGY	2+	2+
8	74	M	CA ESOPHAGUS.	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	4+	3+
9	26	M	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	3+	2+
10	18	M	VARICOCELE	MILD CHRONIC INFLAMMATION	NO SPECIFIC PATHOLOGY	3+	3+
11	68	M	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	2+
12	37	M	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	3+
13	37	F	HYPERSLEENISM	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	2+
14	36	M	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	3+
15	55	M	CA COLON	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	2+
16	68	M	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	1+	3+
17	36	M	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	3+
18	56	M	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	2+
19	51	F	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	3+	2+
20	35	M	VARICOCELE	MILD CHRONIC INFLAMMATION	MILD CHRONIC INFLAMMATION	2+	2+